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APPLICATIONS OF GRANULAR LEGUME INOCULANTS FOR ENHANCED BIOLOGICAL NITROGEN FIXATION

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Application of Granular Legume Inoculants for

Enhanced Biological Nitrogen Fixation

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(a) ABSTRACT/SUMMARY:

Traditionally, legume inoculants have been applied at seeding directly to the seed, either as peat-based powders or as liquid formulations. In recent years, however, manufacturers have begun to develop and formulate *Rhizobium* inoculants as soil-implant granules. This project was initiated in response to a growing interest, and consequent need, for information regarding the efficacy of granular inoculant formulations. Our specific objectives were:

- To evaluate the efficacy of granular inoculants as compared to the more traditional peat and liquid formulations;
- 2) To develop effective delivery strategies for granular inoculants.

Research conducted over a three-year period indicated that N₂ fixation and yield of dryland chickpea and bean, and irrigated bean and field pea, were enhanced by the application of granular inoculants. Typically, granular inoculants were as good as, and often better, than the peat based powder and liquid counterparts. Chickpea, in particular, was very responsive to the granular inoculant formulations. The success of the granular inoculant appears to be related to the promotion of nodulation on lateral roots.

Nodulation of lateral roots is often restricted when pulses are grown on "first time" land; thus, using a granular formulation may be particularly desirable in this situation. The granular products similarly preformed well under conditions of environmental stress.

(b) EXECUTIVE SUMMARY:

Experiments to evaluate the efficacy of granular soil-implant inoculants as compared to peat-based and liquid inoculants applied directly to the seed coat were conducted during the 1997 and 1998 field seasons. Granular inoculants were applied either in the seed row, or placed in a sideband (i.e., 2.5 cm to the side) at depths of either 2.5 or 8 cm below the seed. A total of ten trials, at six different locations were conducted over the two-year period using desi- (var. Miles) and kabuli-type (var. Sanford) chickpea as the host crops. The development and position (i.e., lateral root versus crown region) of chickpea root nodules were evaluated, and N₂ fixation and final seed yield were determined. Mr. Stephen Kyei-Boahen conducted these studies as a component of his Ph.D. studies. Results reported in this document regarding the efficacy of spring-applied inoculants for dryland chickpea represent portions of Mr. Kyei-Boahen's Ph.D. dissertation.

In general, inoculation of chickpea with both clay-based and peat-based granular inoculants proved to be effective at promoting N₂ fixation and yield of chickpea. The benefits of using the granular inoculant apparently were related to the distribution of the nodules on the root system. In particular, granular inoculants promoted nodule development on the lateral roots and data suggest that final seed yields generally were well correlated with the presence of these lateral root nodules. Thus, the data collected throughout this portion of the overall study suggest that pulse crop yields may be improved by promoting sufficient nodulation of the lateral roots. Soil inoculation using granular inoculants can be used to achieve lateral root nodulation because the inoculum can be positioned in the soil zone to target the young developing roots.

The efficacy of granular soil-implant inoculants for dryland bean, and irrigated bean and pea also was evaluated during the 1997 and 1998 field seasons. The irrigated trials were conducted in both 1997 and 1998 in collaboration with Mr. Terry Hogg at the Saskatchewan Irrigation Development Centre (S.I.D.C.), Outlook, SK. The dryland bean trials were conducted at Rosthern and Floral in 1998.

Experiments conducted under both irrigation and dryland conditions support the contention that beans are not efficient N-fixers. Under the irrigated conditions of these experiments, it is likely the relatively high levels of available soil N limited N_2 fixation. However, even when the soil N levels were considerably lower, as was the case for the dryland bean experiments, the levels of N_2 fixation remained relatively low.

Although levels of N₂ fixation were relatively low in the irrigated bean experiments, it is interesting to note that the highest seed yields typically were associated with the granular inoculant treatments, although treatment differences often were not considered statistically significant. These results are in keeping with reports by others who have similarly observed that granular inoculants are as good as, and often better, than liquid and peat-based powders (e.g., Brockwell et al., 1980, 1985).

Data from irrigated field pea trials similarly suggest that inoculation using granular formulations was successful in terms of promoting seed yield. It is important to note, however, that other formulations (i.e., liquid and peat), in some instances, performed as well as the granular products. This is an important observation because it indicates that although granular inoculants were at least as good as other formulations,

they were not necessarily always better than other formulations, and in all situations. Specifically, our results suggest that the granular inoculant formulations often were the "best performers" when plants were grown under environmental stress (i.e., droughty conditions). However, when conditions were ideal (i.e., excellent seedbed and soil moisture conditions) for both crop growth and survival of the *Rhizobium* bacteria, the yield advantages associated with the granular formulations as compared to the liquids and peat based powders, were greatly reduced or undetectable. Thus, given "ideal" conditions, all of the currently available inoculant formulations can be equally capable of promoting similar levels of nodulation and subsequent seed yield.

In general, although placement of the granular products (i.e., sideband versus seed row placement) apparently influenced the success of the inoculation treatment, the effects of placement were inconsistent and unpredictable. Therefore, we concluded that either placement can result in successful nodulation, as long as the band is in close proximity to the emerging root.

Separate experiments also were conducted over a three-year period to investigate the potential of applying granular inoculants directly to the soil, prior to seeding, as a "fall-banded" treatment. Fall banded granular inoculant treatments were compared to spring applied liquid, peat-based powder and granular inoculant treatments. Two experiments were conducted in 1997 using desi-type chickpea as the test crop at Saskatoon and Clavet. These experiments were repeated in 1998 at Young, using chickpea, and dryland bean as the test crops. Two final experiments (i.e., one dryland bean and one chickpea experiment) were conducted in 1999. Inoculants used in the experiments were prepared by MicroBio RhizoGen Corp. (MBR), Agrium Biologicals, and Liphatech Inc. (i.e., commercially available inoculants).

Results from the chickpea studies were encouraging and suggested that fall banding of granular products may result in successful nodulation and N₂ fixation in chickpea. Our results indicated that the chickpea *Rhizobium* used in these experiments were capable of surviving the winter. Moreover, the positioning of the granular inoculant bands at right angles to the seed rows resulted in excellent nodulation in the subsequent chickpea crop. It is important to recognize that these results may not apply for all *Rhizobium* strains and it is urged that caution be used in interpreting the practical significance of these results.

In contrast to the results from the fall banding experiments using chickpea as the test crop, experiments with dry bean indicated that fall application of granular inoculants was unsuccessful. It is not known if the failure of a fall banded granular inoculant treatment was related to the *Rhizobium* strain, the crop, or both. For example, the inoculant failure may have occurred because the bean *Rhizobium* were unable to survive the winter conditions. Alternatively, the rooting pattern of the bean plants may have restricted contact between the inoculant bands applied in the fall at right angles to the seed row, thereby limited nodulation. In contrast, granular inoculants applied in the furrow at seeding may have been better positioned to encourage contact between the emerging bean roots and the inoculant granules.

Although the possibility may exist to promote nodulation of pulses by applying granular inoculants in the fall, inconsistent results (i.e., between sites and with different

crops) suggest that this practice is not risk free. Thus, we have concluded that fall banding of granular inoculants should not be actively promoted as an effective alternative to spring applications without further investigation.

This project generated considerable interest in the farming community and results from this study have been presented at a number of producer meetings across

Saskatchewan. In addition, results of these experiments have been presented at both provincial (i.e., Soils and Crops Workshop) and international (i.e., Agronomy Society of America) meetings. More experiments were ultimately conducted than originally planned due to the generosity of interested parties including the S.I.D.C., MBR, Westco Fertilizers Ltd. and the Crop Development Centre (Dr. Bert Vandenburg). For example, the original proposal indicated that we would conduct a total of four chickpea experiments and two bean experiments to evaluate the efficacy of granular legume inoculants as compared to more traditional peat and liquid formulations. Over the course of this project, and through the financial and "in-kind" assistance provided by other agencies, we expanded this project to include a total of ten chickpea experiments, two pea experiments and four bean experiments, all of which were designed to examine the efficacy of granular inoculants.

(c) TECHNICAL REPORT

Background:

The specific objectives of this research project were:

- To evaluate the efficacy of granular legume inoculants as compared to the more traditional peat and liquid inoculant formulations;
- 2) To develop effective delivery strategies for granular legume inoculants.

Field experiments were initiated in 1997 and were conducted over a three-year period to examine the efficacy of granular legume inoculants and to develop effective delivery strategies for granular legume inoculants. Throughout the course of this project, there has been a considerable amount of collaborative research conducted. In particular S.I.D.C. provided "in-kind" support through a collaborative study investigating the impact of inoculant formulation on N₂-fixation and yield of irrigated pea and bean. This research also has attracted financial support from industry, including MBR and Westco Fertilizers, Ltd.

Project Activities:

1. Effect of Inoculant Formulation and Placement on Biological N2 Fixation by Chickpea

1.1 Materials and Methods

Field experiments were conducted at four sites in Saskatchewan in 1997 (Elbow, Kenaston, Outlook and Watrous) and at two sites in 1998 (Outlook and Watrous). The sites were located in the Dark Brown soil zone within commercial fields testing low in available N levels. There was no history of chickpea production at any of the sites. Soils were sampled prior to seeding and soil characteristics are reported in Table 1. Chickpea grown on samples of the soils obtained from each site did not nodulate after six weeks in a growth chamber pot experiment, confirming the absence of *Rhizobium ciceri*.

Table 1. Soil test data (0-30cm) from the experimental sites, 1997 and 1998.

Locations	Total moisture content (%)	pН	E C (mS cm ⁻¹)*	N (kg ha ⁻¹)	P (kg ha ⁻¹)	K (kg ha ⁻¹)
			1997			
Elbow	9.5	7.9	0.33	8.8	11.2	440
Kenaston	13.1	8.2	0.45	8.4	6.6	240
Outlook	16.4	7.1	0.82	10.8	9.2	540
Watrous	19.4	7.5	0.48 1998	9.2	32.0	540
Outlook	10.6	8.3	0.5	12.4	18.4	440
Watrous	19.4	8.1	0.2	16.4	12.4	540

Desi chickpea (var. Myles) was sown on 1.5 x 12 m plots using a randomized complete block design with four replications at all sites. Planting in 1997 was accomplished on 14 May at Elbow, Kenaston and Outlook, and on 20 May at Watrous. In 1998, planting occurred on 9 and 20 May at Watrous and Outlook, respectively. Plots were seeded using a double disc press drill with separate discs for seed and fertilizer placement (Fabro Ltd., Swift Current, SK) and consisted of 7 rows (six chickpea rows and one flax row) at 15.0 cm spacings. Duplicate (but independent) experiments using kabuli chickpea (var. Sanford) were conducted at Kenaston and Watrous in 1997 and at Outlook and Watrous in 1998. Desi chickpea was seeded at 140 kg ha⁻¹ and kabuli was seeded at 160 kg ha⁻¹.

The experiments were conducted using a randomized complete block design consisting of eleven treatments, replicated four times (Table 2). Initially, the treatments consisted of commercially available inoculants supplied by MicroBio RhizoGen Corp. (MBR), Agrium Biologicals, or Liphatech Inc. and were as follows: 1) seed inoculation using two different peat inoculants (A or B brand) or two different liquid inoculants (A or B brand); 2) soil inoculation, with two granular inoculants (A or B) placed either in the furrow with the seed at planting, side banded 2.5 cm below the seed or 8 cm below the seed and 3) a non-inoculated control. Inoculants with the same designation, e.g., A, indicate that identical *Rhizobium* strain or strains were used in the different carriers. Inoculant A contained a single strain, BCF 32 (MicroBio RhizoGen, Saskatoon, SK) whereas inoculant B contained a mixture of three strains, 27A2, 27A7 and 27A9 (LiphaTec Inc., Milwaukee, W.I). The liquid formulation of inoculant B was not available in 1997; hence an experimental liquid formulation (Inoculant C) containing single strain 27A2 (Agrium Biologicals Inc., Saskatoon, SK) was used.

Table 2. Names, designation and the rates of commercial inoculants used in 1997 and 1998.

Inoculant	Designation	Manufacturer	Application rate
BCF 32 liquid	Liquid A	MicroBio RhizoGen	4.5 ml/kg seed
Agrium liquid	Liquid C*	Agrium Biologicals Inc.	4.5 ml/kg seed
LiphaTec liquid	Liquid B	LiphaTec Inc.	4.5 ml/kg seed
BCF 32 peat	Peat A	MicroBio RhizoGen	1.95 g/kg seed
LiphaTec peat	Peat B	LiphaTec Inc	6.15 g/kg seed
BCF 32 granular	Granular A	MicroBio RhizoGen	9.0 kg/ha
Soil Implant granular	Granular B	LiphaTec Inc	5.6 kg/ha

^{*} Liquid formulation C was used in 1997 instead of Liquid B since it was not available

Seed inoculation was performed by thoroughly mixing a measured amount (according to manufacturer's recommendations) of peat or liquid inoculant with 1.5 kg seed in plastic bags using 5 mL of 1% gum arabic solution as sticker immediately before seeding. The granular inoculants were applied through a separate box connected to side banding discs (adjusted for the various depths). Triple superphosphate (0-45-0) at the rate of 20 kg P₂O₅ ha⁻¹ was applied at planting in the seed row. To avoid contamination, the non-inoculated plots were planted first. In addition, treatments containing the same

Rhizobium strains were planted consecutively before switching to other treatments to minimize the potential for inadvertently contaminating the treatments. Moreover, the seeding equipment was thoroughly cleaned with a vacuum cleaner and then disinfected with 70% ethanol between each treatment.

Mean monthly precipitation and air temperature recorded over the 1997 and 1998 growing seasons for the various sites are presented in Table 3.

Table 3. Mean monthly rainfall and temperature (maximum) data for the experimental locations during the growing season, 1997 and 1998

	Preci	pitation (m	im)	Tempe	rature (°C))
Month	1997	1998	Normal	1997	1998	Normal
			Elbow			
May	26.4	36.2	49.0	17.2	20.4	18.1
June	101.8	78.8	53.3	23.0	20.5	23.0
July	18.4	6.0	56.9	26.6	27.2	26.1
August	46.8	22.4	35.8	26.4	28.9	25.6
			Outlook			
May	18.4	13.0	30.2	17.6	21.5	18.7
June	80.0	111.6	60.4	23.0	21.2	23.3
July	6.2	21.0	54.5	26.2	27.0	25.8
August	35.4	73.4	34.2	26.5	27.8	25.1
			Watrous			
May	65.2	54.8	51.2	17.1	20.0	18.1
June	106.4	147.4	69.1	23.4	19.9	22.7
July	17.6	34.6	59.0	25.4	25.0	25.3
August	64.0	39.6	37.6	25.8	27.5	24.6
			Davidson*			
May	47.0	61.5	40.6	16.7	19.9	18.1
June	70.3	159.1	58.3	23.2	19.5	22.7
July	18.6	68.2	55.8	25.8	25.0	25.8
August	54.9	40.7	38.7	25.6	27.3	25.1

Source: Environment Canada, Saskatoon, SK

In 1997, sampling was performed by randomly excavating the root systems of five plants to a depth of approximately 20 cm from the central rows of each plot at the flowering and early pod-filling stages for desi and at the early pod-filling and late pod-filling stages for kabuli. Soil adhering to the roots was carefully removed and the whole plants and dropped nodules were bagged and transported to the laboratory. Roots were gently washed under running tap water and nodules were collected. Nodules from the crown region and lateral roots were separated and counted. The nodules and the whole plants were dried in an oven at 60 °C for 7 d for dry weight. The crown region was

^{*}data for Kenaston was not available hence data for Davidson (nearest station) is presented.

defined as that part of the root extending 3 cm in all directions from the stem base whereas the lateral roots were defined as that part of the root system extending beyond 3 cm from the stem base.

At crop maturity, a one-meter section of center rows from each plot was handharvested for seed yield determination. Nitrogen fixation was determined using the natural ¹⁵N abundance method (Rennie and Kemp, 1984).

Data collection and analysis for the 1998 experiments were similar to the previous year except for the first sampling of the desi plots, which was done at the early pod filling stage instead of flowering stage. In addition, the plants were not sampled at the late pod-filling stage at the Outlook site because it was not possible to recover most of the nodules since the soil was too dry and hard to excavate effectively.

Data for each site were analyzed separately using the general linear model procedure of the SAS system (SAS Institute, Cary, NC). In addition, combined analyses were conducted separately for the 1997 and 1998 experiments. Significant differences between treatment means were evaluated with LSD at the 5% probability level. Orthogonal contrasts ($P \le 0.05$) were used to statistically compare inoculant formulations and inoculation methods. Combined analysis of data over years was not performed because although four similar experiments were conducted at four locations in 1997, only two experiments, each at each location were conducted for desi in 1998. For the kabuli, although two similar experiments were conducted in both years, one of the 1998 experiments was conducted at a site different from that of 1997. Moreover, liquid B was not available in 1997 so liquid C was used instead; therefore, one of the treatments was different between years. Regression analyses of shoot dry matter per plant and seed yield per ha averaged over sites were performed separately on dry weight of crown and lateral root nodules per plant average over sites.

1.2 Results of the Inoculant Formulation Trials for Chickpea

1.2.1 Plant growth and nodulation

Moisture conditions at Watrous in both 1997 and 1998 favoured early seedling emergence, and plant growth was more vigorous than at the other sites. However, plant growth at Elbow in 1997 was restricted by low soil moisture at seeding (Table 1) but this apparently did not affect plant growth response to inoculation. On the other hand, in 1998, seeding at Outlook was eleven days later than at Watrous due to drought conditions, but no rain occurred during this delay. The Outlook plots were seeded on 20 May and according to Environment Canada, average precipitation at Outlook for May 1998 was 57% less than normal (Table 3). As a result of the low soil moisture (Table 1), seedling emergence was slow and plant stand was limited, particularly in treatments where granular inoculants were placed below the seed. The soil was very dry and it was observed that the upper 30 cm was very hard and difficult to penetrate. In fact, the resistance encountered by the disc openers for both the granular inoculant and the seed forced the seeds to be deposited just below the soil surface where the soil moisture content was believed to be too low for optimum germination, particularly for the large-seeded kabuli. Dry conditions during the later part of the growing season at Outlook in

1998 also made sampling for plant roots and attached nodules difficult and plans to sample roots at late pod filling were abandoned. Inoculation treatments produced similar results for both desi and kabuli chickpeas at all locations (Tables 4-19). Therefore, genotype data were averaged over locations for each year. Except for the Outlook plots in 1997 (Table 4 and 5), limited (though sparse) nodulation occurred on non-inoculated plots despite the care taken to avoid contamination.

Inoculation treatments and depth of inoculant placement significantly influenced nodulation and nodulation patterns in both chickpea genotypes at all locations (Tables 4-19). For the 1997 growing season, average over locations, the peat-based inoculants produced more nodules per plant than for the liquid and granular formulated inoculants at both sampling dates in both desi (Tables 12 and 13) and kabuli chickpea (Tables 14 and 15). In the desi experiments, the differences in total nodule numbers for the liquid vs. peat inoculant and seed-applied vs. soil inoculation (liquid + peat vs. granular) treatments were significant at the 5% level at the flowering stage. However, as the plants approached early pod-filing stage, the differences increased and were significant at the 1 % probability level. A similar trend was found for the contrast analysis of total nodule numbers for the granular inoculant placed with the seed vs. placement below the seed. In this case, the total nodule numbers for the granular inoculants applied in the seed furrow were significantly higher than when the granular inoculants were placed below the seed. However, no significant differences in total nodule numbers were observed between the granular inoculant placed in the seed furrow and the peat-based inoculant. The data indicated that rhizobia strain or strains in the same formulation did not differ significantly and there was no strain interaction. Furthermore there was no significant differences between granular inoculant placed 2.5 cm and 8.0 cm below the seed.

In contrast to the other desi sites, the liquid formulated inoculant treatments at the Watrous site produced relatively more nodules (Tables 8 and 9). Generally, total nodule numbers at both flowering and early pod-filling stages for desi at Outlook (Tables 4 and 5), Kenaston (Tables 6 and 7) and Watrous locations (Tables 8 and 9) were generally two to three and half times greater than that recorded at Elbow (Tables 10 and 11).

Table 4. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage, at the Outlook location, 1997.

	No	dule no. pl	ant-1	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt.	
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)	
Non-inoculated	0	0	0	0	0	0	1.23	
Liquid A	0.60	4.80	5.40	12.0	11.5	23.5	1.24	
Liquid C	0.45	5.25	5.70	1.5	8.5	10.0	1.07	
Peat A	1.50	4.40	5.90	11.0	9.0	20.0	1.25	
Peat B	3.00	6.80	9.80	18.5	19.0	37.5	1.04	
Gran A ws	1.70	1.70	3.40	12.0	10.0	22.0	1.35	
Gran A 2.5 cm	0.15	4.60	4.75	4.5	34.0	38.5	1.40	
Gran A 8.0 cm	0.70	3.50	4.20	11.0	27.0	38.0	1.15	
Gran B ws	2.25	8.35	10.60	14.0	26.5	40.5	1.05	
Gran B 2.5 cm	0.35	4.05	4.40	3.5	38.5	41.5	1.40	
Gran B 8.0 cm	0.45	4.35	4.80	8.5	41.5	50.0	1.49	
LSD _(0.05)	1.55	5.24	5.66	11.1	18.9	25.3	0.33	

Gran = granular, ws = with seed.

Table 5. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at the Outlook, location, 1997.

	Noc	dule no. p	lant ⁻¹	Nodule	dry wt. (m	ng plant ⁻¹)	Shoot dry wt.	
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)	
Non-inoculated	0	0	0	0	0	0	3.70	
Liquid A	1.05	0.85	1.90	26.0	31.0	57.0	4.14	
Liquid C	2.10	1.90	4.00	37.0	24.0	61.0	4.34	
Peat A	4.55	2.85	7.40	88.5	52.5	141.0	4.87	
Peat B	2.65	1.80	4.45	72.5	19.5	92.0	4.78	
Gran A ws	1.30	2.40	3.70	46.5	45.5	92.0	4.92	
Gran A 2.5 cm	0.65	1.80	2.45	44.5	88.0	132.5	5.66	
Gran A 8.0 cm	0	2.65	2.65	0	125.5	125.5	4.90	
Gran B ws	1.95	2.65	4.60	68.0	47.5	115.5	4.54	
Gran B 2.5 cm	1.55	2.15	3.70	79.5	90.0	169.5	5.36	
Gran B 8.0 cm	0.60	3.25	3.85	16.5	108.0	124.5	6.05	
LSD _(0.05)	1.56	1.22	2.47	65.7	55.0	84.7	1.44	

Gran = granular, ws = with seed.

Table 6. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage at the Kenaston location, 1997.

	No	dule no. pl	ant ⁻¹	Nodule	dry wt (m	g plant ⁻¹)	Shoot dry wt.	
Inoculant	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)	
Non-inoculated	0.10	3.60	3.70	2.0	8.5	10.5	1.39	
Liquid A	1.05	2.45	3.50	12.0	15.0	27.0	1.39	
Liquid C	1.30	2.30	3.60	16.5	16.5	33.0	1.31	
Peat A	2.50	3.80	6.30	53.0	42.0	95.0	1.47	
Peat B	4.80	2.00	6.80	81.0	16.5	97.5	1.61	
Gran A ws	1.30	4.15	5.45	29.5	67.5	97.0	1.82	
Gran A 2.5 cm	0.50	4.35	4.85	6.0	70.0	76.0	2.03	
Gran A 8.0 cm	0.40	2.45	2.85	8.0	75.5	83.5	1.87	
Gran B ws	1.85	4.95	6.80	24.5	41.5	66.0	1.54	
Gran B 2.5 cm	0.65	3.85	4.50	21.5	67.5	89.0	1.61	
Gran B 8.0 cm	0.35	2.25	2.60	9.0	42.0	51.0	1.90	
LSD _(0.05)	1.30	2.94	3.19	28.3	40.6	41.5	0.41	

Gran = granular, ws = with seed.

Table 7. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at the Kenaston location, 1997.

	Nodule	no. plant		Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt.
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0.20	0.35	0.55	10.0	26.0	36.0	2.96
Liquid A	1.95	1.00	2.95	39.5	14.0	53.5	2.52
Liquid C	2.55	1.15	3.70	75.5	25.0	100.5	2.63
Peat A	3.65	1.20	4.85	120.5	38.5	159.0	3.69
Peat B	5.10	2.05	7.15	112.5	39.0	151.5	3.17
Gran A ws	1.95	1.50	3.45	53.0	45.0	98.0	2.51
Gran A 2.5 cm	0.40	2.05	2.45	15.0	98.5	113.5	3.79
Gran A 8.0 cm	0.25	1.00	1.25	2.5	29.5	32.0	3.65
Gran B ws	2.60	2.75	5.35	51.0	56.0	107.0	3.74
Gran B 2.5 cm	0.30	2.50	2.80	5.0	105.5	110.5	3.52
Gran B 8.0 cm	0.15	1.75	1.90	11.5	106.5	118.0	4.73
LSD _(0.05)	1.40	1.41	2.05	45.2	60.2	72.3	0.83

[†] Gran = granular, ws = with seed.

Table 8. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage at the Watrous location, 1997.

	Nodu	le no. pla	nt ⁻¹	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt.
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0.45	0.65	1.10	6.0	1.5	7.5	1.43
Liquid A	3.60	2.20	5.80	47.0	13.0	60.0	1.70
Liquid C	2.45	4.30	6.75	68.5	16.0	84.5	1.34
Peat A	2.35	2.95	5.30	44.5	22.0	66.5	1.28
Peat B	2.65	3.45	6.10	54.5	20.0	74.5	1.63
Gran A ws	1.85	2.25	4.10	34.0	27.0	61.0	1.71
Gran A 2.5 cm	0.55	3.35	3.90	21.0	50.0	71.0	1.33
Gran A 8.0 cm	0.25	2.00	2.25	9.0	30.0	39.0	1.35
Gran B ws	0.90	2.75	3.65	19.0	35.0	54.0	1.46
Gran B 2.5 cm	0.65	3.15	3.80	26.5	58.0	84.5	1.45
Gran B 8.0 cm	0.35	2.30	2.65	5.5	25.5	31.0	1.23
LSD _(0.05)	1.46	1.86	2.38	41.3	28.3	48.2	0.40

[†] Gran = granular, ws = with seed.

Table 9. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at the Watrous location, 1997.

	Nodu	le no. pla	nt ⁻¹	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt.	
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)	
Non-inoculated	0.55	1.45	2.00	12.0	30.0	42.0	4.74	
Liquid A	5.80	3.90	9.70	106.0	46.5	152.5	5.74	
Liquid C	7.10	1.75	8.85	144.0	29.5	173.5	5.25	
Peat A	4.75	2.30	7.05	102.5	60.5	163.0	5.02	
Peat B	6.00	2.25	8.25	135.5	26.5	162.0	4.59	
Gran A ws	4.00	4.05	8.05	114.0	73.0	187.0	6.33	
Gran A 2.5 cm	0.75	3.75	4.50	7.5	150.0	157.5	6.66	
Gran A 8.0 cm	1.25	3.95	5.20	22.0	110.0	132.0	5.86	
Gran B ws	3.55	6.85	10.40	161.0	96.0	257.0	7.02	
Gran B 2.5 cm	0.55	4.00	4.55	19.5	243.5	263.0	6.25	
Gran B 8.0 cm	1.40	4.00	5.40	85.0	127.5	212.5	6.33	
LSD(0.05)	2.14	2.46	3.02	89.4	58.9	88.7	1.83	

† Gran = granular, ws = with seed.

Table 10. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage at the Elbow location, 1997.

	Nod	ule no. pl	ant ⁻¹	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt.	
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)	
Non-inoculated	0.05	0.10	0.15	1.0	1.5	2.5	0.88	
Liquid A	0.25	0.25	0.50	2.5	1.0	3.5	0.81	
Liquid C	0.50	1.25	1.75	5.0	5.5	10.5	1.04	
Peat A	1.05	1.20	2.25	26.5	8.5	35.0	0.87	
Peat B	1.65	1.05	2.70	21.5	8.0	29.5	0.88	
Gran A ws	0.55	0.75	1.30	10.0	3.5	13.5	0.84	
Gran A 2.5 cm	0.40	1.00	1.40	11.5	15.0	26.5	0.87	
Gran A 8.0 cm	0	0.50	0.50	0	13.5	13.5	0.93	
Gran B ws	1.25	2.00	3.25	16.0	13.0	29.0	0.89	
Gran B 2.5 cm	0.65	1.65	2.30	21.0	24.5	45.5	0.97	
Gran B 8.0 cm	0.30	1.30	1.60	3.5	8.0	11.5	0.97	
LSD _(0.05)	0.84	1.20	1.52	16.4	11.3	22.2	ns	

* Gran = granular, ws = with seed.

Table 11. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at the Elbow, location, 1997.

	Nodule	no. plant	1	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt.	
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)	
Non-inoculated	0.15	0.40	0.55	4.5	14.5	19.0	3.45	
Liquid A	0.30	0.30	0.60	19.5	15.0	34.5	3.08	
Liquid C	0.45	0.65	1.10	6.0	9.0	15.0	3.88	
Peat A	2.10	0.70	2.80	40.5	18.0	58.5	3.00	
Peat B	3.50	1.15	4.65	80.0	35.0	115.0	3.45	
Gran A ws	0.75	1.00	1.75	10.0	42.0	52.0	3.68	
Gran A 2.5 cm	0.55	1.40	1.95	22.5	61.5	84.0	3.44	
Gran A 8.0 cm	0	0.70	0.70	0	48.5	48.5	3.74	
Gran B ws	1.80	1.20	3.00	50.0	21.0	71.0	3.59	
Gran B 2.5 cm	0.70	1.05	1.75	16.0	44.0	60.0	3.58	
Gran B 8.0 cm	0.30	1.40	1.70	5.5	77.5	83.0	4.02	
LSD _(0.05)	0.95	0.73	1.26	20.8	38.0	6.7	ns	

† Gran = granular, ws = with seed.

Table 12. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage, averaged over the Elbow, Kenaston, Outlook and Watrous locations, 1997.

	Nodu	ile no. pla	nt ⁻¹		ule dry wi g plant ⁻¹)	t.	Shoot dry wt.
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoc	0.15	1.09	1.24	2.3	2.9	5.2	1.23
Liq A	1.38	2.43	3.80	18.4	10.1	28.5	1.29
Liq C	1.18	3.28	4.45	22.9	11.6	34.5	1.19
Peat A	1.85	3.09	4.94	33.8	20.4	54.2	1.22
Peat B	3.03	3.33	6.35	43.9	15.9	59.8	1.29
Gran A with seed	1.35	2.21	3.56	21.4	27.0	48.4	1.43
Gran A 2.5 cm bs	0.40	3.33	3.73	10.8	42.3	53.0	1.41
Gran A 8.0 cm bs	0.34	2.11	2.45	7.0	36.5	43.5	1.33
Gran B with seed	1.56	4.51	6.08	18.4	29.0	47.4	1.24
Gran B 2.5 cm bs	0.58	3.18	3.75	18.1	47.0	65.1	1.36
Gran B 8.0 cm bs	0.36	2.55	2.91	6.6	29.3	35.9	1.40
LSD _(0.05)	0.99	1.69	1.98	19.0	15.7	23.9	ns
Contrasts							
Non-inoc vs. inoc‡	1.05**	1.91**	2.96**	17.8 *	24.0**	41.8**	0.09
Liq vs. peat	1.16**	0.36	1.52*	18.2**	7.3	25.5**	0.02
Liq A vs. liq C	0.2	0.85	0.65	4.5	1.5	6.0	0.10
Peat A vs. peat B	1.18*	0.24	1.41	10.1	4.5	5.6	0.07
Liq+peat vs. gran	1.10**	0.05	1.14*	16.0**	20.7**	4.6	0.11*
Gran ws vs. gran bs	1.04**	0.57	1.61*	9.3	10.8*	1.5	0.04
Gran 2.5 vs. gran 8.0	0.14	0.93	1.06	7.7	11.8*	19.4*	0.02
Gran A vs. gran B	0.14	0.86	1.00	1.3	0.2	1.2	0.06
Gran str x ws vs. bs	0.02	0.15	0.13	6.1	7.2	13.2	0.08
Gran str x 2.5 vs. 8.0	0.08	0.30	0.22	3.9	6.0	9.9	0.06

ns, *, ** Non-significant, significant at 0.05 and 0.01 levels, respectively.

[‡] Difference

Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain

Table 13. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage, averaged over the Elbow, Kenaston, Outlook and Watrous locations, 1997.

	Nodule	no. plan	t ⁻¹		ule dry w		Shoot dry wt.
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant-1)
Non-inoc	0.23	0.55	0.78	6.6	17.6	24.2	3.71
Liq A	2.28	1.51	3.79	47.8	26.6	74.4	3.87
Liq C	3.05	1.36	4.41	65.6	21.9	87.5	4.02
Peat A	3.76	1.76	5.53	88.0	42.4	130.4	4.14
Peat B	4.31	1.81	6.13	100.1	30.0	130.1	4.00
Gran A with seed	2.00	2.24	4.24	55.9	51.4	107.3	4.36
Gran A 2.5 cm bs	0.59	2.25	2.84	22.4	99.5	121.9	4.88
Gran A 8.0 cm bs	0.38	2.08	2.45	6.1	78.4	84.5	4.54
Gran B with seed	2.48	3.36	5.84	82.5	55.1	137.6	4.72
Gran B 2.5 cm bs	0.78	2.42	3.20	30.0	120.8	150.8	4.68
Gran B 8.0 cm bs	0.61	2.60	3.21	29.6	104.9	134.5	5.28
LSD _(0.05)	1.51	1.08	1.87	40.7	39.8	46.2	0.71
Contrasts							
Non-inoc vs. inoc‡	1.79**	1.59**	3.38**	46.2**	45.5**	91.7**	0.74**
Liq vs. peat	1.37**	0.35	1.73**	37.4**	12.0	49.3**	0.13
Liq A vs. liq C	0.77	0.15	0.62	17.8	4.7	13.1	0.15
Peat A vs. peat B	0.55	0.05	0.60	12.1	12.4	0.3	0.14
Liq+peat vs. gran	2.21**	0.88**	1.33**	37.6**	54.8**	17.2	0.74**
Gran ws vs. gran bs	1.65**	0.46	2.11**	47.2**	47.7**	0.5	0.31
Gran 2.5 vs. gran 8.0	0.19	0	0.19	8.3	18.5	26.9	0.13
Gran A vs. gran B	0.30	0.60*	0.91	19.2	17.2	36.4**	0.30
Gran str x ws vs. bs	0.03	0.38	0.41	3.3	11.1	7.8	0.21
Gran str x 2.5 vs. 8.0	0.03	0.18	0.20	8.0	2.6	10.6	0.47

*, ** Significant at 0.05 and 0.01 levels, respectively.

Difference.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 14. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stages, averaged over the Kenaston and Watrous locations, 1997.

	Nodul	e no. plan	t ⁻¹	Nodule d	lry wt. (mg	plant ⁻¹)	Shoot dry wt.
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g pl ⁻¹)
Non-inoc	0.45	0.43	0.88	9.5	13.0	22.5	6.18
Liq A	2.60	2.00	4.60	64.3	58.0	122.2	6.24
Liq C	3.90	1.98	5.88	80.3	53.5	133.8	6.14
Peat A	5.73	3.03	8.75	178.8	67.0	245.8	6.55
Peat B	7.33	4.20	11.53	221.3	88.0	309.3	8.14
Gran A with seed	2.93	5.53	8.45	82.0	116.3	198.3	7.32
Gran A 2.5 cm bs	0.50	5.48	5.98	20.3	174.3	194.5	8.94
Gran A 8.0 cm bs	0.40	5.33	5.73	4.3	122.8	127.0	8.25
Gran B with seed	2.25	5.55	7.80	38.0	123.0	161.0	7.69
Gran B 2.5 cm bs	0.75	4.35	5.10	22.5	162.5	185.0	8.83
Gran B 8.0 cm bs	0.43	4.70	5.13	28.3	184.8	213.0	8.64
LSD _(0.05)	1.65	3.19	3.60	71.5	47.1	75.8	1.49
Contrasts							
Non-inoc vs. inoc‡	2.23**	3.79**	6.02**	64.5*	102.0**	166.5**	1.49*
Liq vs. peat	3.28**	1.63	4.90**	127.8**	21.8	149.6**	1.16*
Liq A vs. liq C	1.30	0.02	1.28	16.0	4.5	11.6	0.10
Peat A vs. peat B	1.60	1.17	2.78	42.5	21.0	63.5	1.59*
Liq+peat vs. gran	3.68**	2.35**	1.33	103.6**	80.7**	23.0	1.51**
Gran ws vs. gran bs	2.07**	0.58	2.64*	41.2	41.5**	0.2	1.16*
Gran 2.5 vs. gran 8.0	0.21	0.10	0.11	5.1	14.6	19.8	0.44
Gran A vs. gran B	0.13	0.58	0.71	5.9	19.0	13.1	0.22
Gran str x ws vs. bs	0.37	0.07	0.29	18.1	7.5	25.6	0.17
Gran str x 2.5 vs. 8.0	0.11	0.25	0.14	10.9	36.9*	47.8	0.25

*, ** Significant at 0.05 and 0.01 levels, respectively.

Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Difference.

g pl = g plant

Table 15. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the late pod-filling stages, averaged over the Kenaston and Watrous locations, 1997.

	Nodule	no. plant	-1	Nodule di	ry wt. (mg p	olant ⁻¹)	Shoot dry wt.
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g pl-1)
Non-inoc	0.48	0.50	0.98	18.0	33.0	51.0	8.77
Liq A	2.25	2.58	4.83	67.8	77.0	144.8	10.30
Liq C	3.58	2.23	5.80	104.8	123.5	228.3	9.16
Peat A	6.00	4.35	10.35	175.3	105.5	280.8	12.31
Peat B	5.30	5.45	10.75	184.8	136.5	321.3	12.54
Gran A with seed	2.95	5.38	8.33	76.8	154.0	230.8	12.49
Gran A 2.5 cm bs	0.78	7.03	7.80	34.0	179.8	213.8	15.57
Gran A 8.0 cm bs	0.15	4.98	5.13	2.3	178.3	180.5	14.20
Gran B with seed	1.93	5.75	7.68	42.5	145.0	187.5	12.12
Gran B 2.5 cm bs	0.48	4.75	5.23	19.8	204.3	224.0	13.46
Gran B 8.0 cm bs	0.13	4.03	4.15	9.5	169.8	179.3	15.34
LSD _(0.05)	1.59	3.77	4.54	57.4	91.4	118.0	4.18
Contrasts							
Non-inoc vs. inoc‡	1.87**	4.15**	6.03**	53.7*	114.4**	168.1**	3.98*
Liq vs. peat	2.34**	2.50	5.24**	93.8**	20.8	114.5*	2.70*
Liq A vs. liq C	1.33	0.35	0.97	37.0	46.5	83.5	1.14
Peat A vs. peat B	0.70	1.10	0.40	9.5	31.0	40.5	0.23
Liq+peat vs. gran	3.21**	1.67	1.55	102.4**	61.2**	41.2	2.79**
Gran ws vs. gran bs	2.06**	0.37	2.43	43.3*	33.6	9.8	2.34
Gran 2.5 vs. gran 8.0	0.49	1.39	1.88	21.0	18.0	39.0	0.26
Gran A vs. gran B	0.45	0.95	1.40	13.8	2.3	11.4	0.45
Gran str x ws vs. bs	0.67	0.80	1.47	25.4	15.0	40.4	0.05
Gran str x 2.5 vs. 8.0	0.14	0.67	0.80	10.7	16.5	5.7	1.63

^{*, **} Significant at 0.05 and 0.01 levels, respectively.

^{*}Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Difference.

g pl = g plant-1

In the kabuli experiments, significant differences (P=0.01) in total nodule numbers were also observed between the liquid and peat-based inoculant treatments at both early and late pod-filling stages but differences between the seed-applied and soil applied inoculants (Liquid + peat vs. granular) were not significant (Tables 14 and 15). As in the desi, the total nodule number for the granular inoculant placed in the seed row was significantly higher than that for granular inoculant placed below the seed in the kabuli experiments but the differences were significant only at the early pod-filling stage. The highest nodule numbers per plant in the kabuli experiments were observed at Watrous for peat B (12.8) at the early pod-filling stage (Table 16) and peat A (12.6) at the late pod-filling stage (Table 17). Nodulation in the kabuli at both Watrous and Kenaston locations was similar (Tables 16 to 19).

Table 16. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage at Watrous location, 1997.

	Nodu	le no. plai	nt ⁻¹	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt
Inoculant	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0.50	0.45	0.95	5.5	18.0	23.5	7.74
Liquid A	2.80	2.30	5.10	70.5	42.0	112.5	7.62
Liquid C	5.20	2.50	7.70	103.5	34.0	137.5	7.25
Peat A	7.00	3.65	10.65	138.5	53.0	191.5	7.26
Peat B	7.55	5.20	12.75	180.0	79.5	259.5	9.51
Gran A ws	2.65	7.75	10.40	48.5	119.5	168.5	7.71
Gran A 2.5 cm	0.40	4.20	4.60	32.0	164.0	196.0	8.94
Gran A 8.0 cm	0.30	6.05	6.35	2.0	128.5	130.5	8.94
Gran B ws	2.80	6.30	9.10	48.5	95.0	143.5	8.61
Gran B 2.5 cm	0.95	4.15	5.10	35.5	165.0	200.5	9.56
Gran B 8.0 cm	0.75	3.35	4.10	29.0	143.5	172.5	9.16
LSD _(0.05)	2.65	3.09	3.98	88.5	62.9	105.6	ns

Gran = granular, ws = with seed.

Table 17. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the late pod-filling stage at Watrous location, 1997.

	Node	ule no. pla	nt ⁻¹	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt.	
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)	
Non-inoculated	0.20	0.75	0.95	4.0	16.0	20.0	10.64	
Liquid A	3.15	3.40	6.55	70.0	56.5	126.5	12.93	
Liquid C	2.90	2.00	4.90	51.0	47.5	98.5	9.00	
Peat A	7.05	5.50	12.55	171.0	105.5	276.5	14.16	
Peat B	5.80	4.40	10.20	185.5	67.5	253.0	14.31	
Gran A ws	3.25	6.30	9.55	68.0	82.5	150.5	13.65	
Gran A 2.5 cm	0.80	4.90	5.70	44.0	147.0	191.0	17.09	
Gran A 8.0 cm	0.25	4.40	4.65	2.0	170.5	172.5	15.24	
Gran B ws	2.30	3.50	5.80	55.0	67.5	122.5	10.24	
Gran B 2.5 cm	0.25	3.80	4.05	14.0	178.0	192.0	14.27	
Gran B 8.0 cm	0.25	2.55	2.80	19.0	120.5	139.5	14.64	
LSD _(0.05)	1.68	2.11	3.20	60.3	67.6	88.0	3.56	

† Gran = granular, ws = with seed

Table 18. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage at Kenaston location, 1997.

	Nod	ule no. pla	ant ⁻¹	Nodule	dry wt. (n	ng plant-1)	Shoot dry wt.
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0.40	0.40	0.80	13.5	8.0	21.5	4.63
Liquid A	2.40	1.70	4.10	58.0	74.0	132.0	4.87
Liquid C	2.60	1.45	4.05	57.0	73.0	130.0	5.03
Peat A	4.45	2.40	6.85	219.0	81.0	300.0	5.84
Peat B	7.10	3.20	10.30	262.5	96.5	359.0	6.76
Gran A ws	3.20	3.30	6.50	115.0	113.0	228.0	6.93
Gran A 2.5 cm	0.60	6.75	7.35	8.5	184.5	193.0	8.95
Gran A 8.0 cm	0.50	4.60	5.10	6.5	117.0	123.5	7.55
Gran B ws	1.70	4.80	6.50	27.5	151.0	178.5	6.77
Gran B 2.5 cm	0.55	4.55	5.10	9.5	160.0	169.5	8.09
Gran B 8.0 cm	0.10	6.05	6.15	21.5	226.0	253.5	8.11
LSD _(0.05)	1.99	2.51	3.61	83.3	97.2	139.0	1.97

Gran = granular, ws = with seed.

Table 19. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the late pod-filling stage at Kenaston location, 1997.

	Nod	lule no. pla	ant ⁻¹	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt.
Inoculant	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0.75	0.25	1.00	32.0	50.0	82.0	6.91
Liquid A	1.35	1.75	3.10	65.5	97.5	163.0	7.66
Liquid C	4.25	2.45	6.70	158.5	199.5	358.0	9.32
Peat A	4.95	3.20	8.15	179.5	105.5	285.0	10.45
Peat B	4.80	6.50	11.30	184.0	205.5	389.5	10.77
Gran A ws	2.65	4.45	7.10	85.5	225.5	311.0	11.33
Gran A 2.5 cm	0.75	9.15	9.90	24.0	212.5	236.5	14.05
Gran A 8.0 cm	0.05	5.55	5.60	2.5	186.0	188.5	13.15
Gran B ws	1.55	8.00	9.55	30.0	222.5	252.5	14.00
Gran B 2.5 cm	0.70	5.70	6.40	25.5	230.5	256.0	12.65
Gran B 8.0 cm	0	5.50	5.50	0	219.0	219.0	16.05
LSD _(0.05)	1.12	1.90	2.28	73.1	103.6	138.2	3.02

Gran = granular, ws = with seed.

For the 1997, nodule dry weight often did not follow the same trends as data for the number of nodules produced in both desi and kabuli experiments at all locations (e.g., Tables 4-19). In the desi chickpea, evaluated at early pod-filling, granular inoculants placed 2.5 cm below the seed produced low numbers of nodules, but the total dry weights were not significantly different from that for the peat inoculants (Table 13). Orthogonal contrast of liquid + peat vs. granular inoculant treatment detected no significant differences in total nodule dry weight.

In general, total nodule dry weights for the liquid inoculants were lower than that for the peat and granular inoculants at both flowering and early pod-filling stages in desi chickpea (Tables 12 and 13). At the flowering stage, total nodule dry weight for the granular inoculant placed 2.5 cm was significantly (P = 0.03) higher as compared to that for the 8.0 cm placement. Comparison of total nodule dry weights for granular A and B inoculants at the early pod-filling in the desi showed that granular B was significantly (P = 0.01) better than granular A. The greatest nodule dry weight in the desi experiments (263 mg plant⁻¹) occurred in granular B inoculant placed 2.5 cm below the seed at Watrous at the early pod-filling stage (Table 9).

For the kabuli, total nodule dry weight averaged over locations, was significantly higher for the peat B treatment than the other treatments except for peat A and granular B placed 8 cm below the seed at the early pod-filling stage (Table 14). The same trend was observed at the late pod filling stage where the peat inoculants were, on average, 52, 35 and 33% greater in total nodule dry weight as compared to liquid A, granular A and B placed below the seed respectively (Table 15). However, orthogonal contrast indicated significant differences only between the liquid and peat-based inoculant treatments at the early and late pod-filling stages. The greatest nodule dry weight recorded in the kabuli experiment was 389.5 mg plant⁻¹ for peat B at Kenaston during the late pod-filling stage

(Table 19). Contrast analysis indicated that the interaction between inoculation treatment and location was not significant for total nodule dry weight for both chickpea genotypes at both sampling dates, except for desi at the flowering stage due to the low soil moisture at Elbow at seeding.

Unlike the 1997 field season, the 1998 results showed that the granular inoculants produced more nodules than both the peat and the liquid inoculants; however, the total dry weight of the nodules were generally greater in the peat than the other inoculation treatments in both chickpea genotypes (Tables 20 and 21). For the desi experiments however, soil inoculation typically resulted in significantly higher nodule dry matter than the seed-applied inoculant treatments (Table 20). Liquid A performed poorly in terms of nodulation and was not significantly different from the non-inoculated control in both nodule number and dry weight at the early pod-filling stage. Although total nodule dry weight for liquid B was low, it differed significantly from only that for peat A and the granular inoculant placed with the seed.

Averaged over locations, granular inoculant placed with the desi seed produced 16 and 30% more total nodule dry matter than when the inoculants were placed below the seed for granular A and B, respectively (Table 20 and 21). Total nodule dry weight was greatest in the peat A treatment (307.5 mg plant⁻¹) at Outlook (Table 22) whereas granular A placed 8 cm below the seed recorded the highest nodule dry weight (275 mg plant⁻¹) at Watrous (Table 23). Sampling at the late pod-filling stage was performed only at the Watrous location due to dry soil conditions at Outlook, which made it difficult to excavate and recover roots, and attached nodules. Although total nodule numbers and dry weights obtained at Watrous were low compared to that observed at early pod filling, they followed a similar trend (Table 24).

Table 20. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage, averaged over the Outlook and Watrous locations, 1998.

	Nodu	le no. plar	nt ⁻¹	Nodule d	ry wt. (mg	plant ⁻¹)	Shoot dry wt.
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g pl-1)
Non-inoc	0	0.13	0.13	0	3.0	3.0	4.70
Liq A	0.18	0.35	0.53	9.5	16.0	25.5	4.16
Liq B	2.00	1.40	3.40	76.3	48.0	124.3	4.73
Peat A	3.27	2.38	5.65	193.3	70.8	264.0	5.13
Peat B	3.88	2.30	6.18	150.5	41.0	191.5	5.39
Gran A with seed	2.43	5.45	7.88	80.3	163.0	243.3	6.26
Gran A 2.5 cm bs	1.13	6.23	7.35	21.8	182.3	204.0	5.94
Gran A 8.0 cm bs	0.40	6.70	7.10	7.8	196.3	204.0	7.03
Gran B with seed	2.13	4.70	6.83	87.3	134.5	221.8	5.59
Gran B 2.5 cm bs	1.08	6.10	7.18	25.5	131.5	157.0	5.91
Gran B 8.0 cm bs	0.60	4.95	5.55	19.0	134.5	153.5	6.33
LSD _(0.05)	1.72	1.92	3.03	45.9	76.2	94.3	0.97
Contrasts							
Non-inoc vs. inoc [‡]	1.71*	3.93**	5.64**	67.1*	108.8**	175.9**	0.95**
Liq vs. peat	2.49**	1.47**	3.95**	129.0**	23.9	152.9**	0.82*
Liq A vs. liq B	1.82*	1.05	2.87	66.8**	32.0	98.8*	0.57
Peat A vs. peat B	0.61	0.08	0.53	42.8	29.8	72.5	0.26
Liq+peat vs. gran	1.04**	4.08**	3.04**	67.1**	113.1**	45.9*	1.32**
Gran ws vs. gran bs	1.48**	0.92	0.56	65.3**	12.4	52.9	0.38
Gran 2.5 vs. gran 8.0	0.61	0.34	0.94	10.3	8.5	1.8	0.76*
Gran A vs. gran B	0.05	0.88	0.92	7.3	47.0*	39.7	0.47
Gran str x ws vs. bs	0.50	0.48	0.98	4.5	3.8	8.3	0.28
Gran str x 2.5 vs. 8.0	0.13	0.81	0.69	3.8	5.5	1.8	0.34

*, ** Significant at 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Difference.

[§] g pl = g plant⁻¹

Table 21. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage, averaged over the Outlook and Watrous locations, 1998.

	Nodu	le no. pla	nt ⁻¹	Nodule d	lry wt. (m	g plant ⁻¹)	Shoot dry wt.
Inoculant [†]	Crown	Lateral	Total	Crown	Lateral	Total	(g pl-1)8
Non-inoc	0.05	0.50	0.55	1.5	21.5	23.0	6.53
Liq A	0.55	1.13	1.68	26.5	50.8	77.3	7.78
Liq B	2.45	2.73	5.18	69.3	73.0	142.3	6.39
Peat A	3.25	2.68	5.93	144.0	90.3	234.3	8.39
Peat B	4.88	3.25	8.13	165.0	79.3	244.3	9.30
Gran A with seed	2.50	5.05	7.55	59.0	115.0	174.0	7.86
Gran A 2.5 cm bs	0.88	8.38	9.25	16.0	164.5	180.5	8.86
Gran A 8.0 cm bs	0.58	7.90	8.48	8.8	173.0	181.8	8.57
Gran B with seed	3.00	5.10	8.10	92.3	150.8	243.0	8.97
Gran B 2.5 cm bs	1.40	6.00	7.40	35.3	133.5	168.8	9.02
Gran B 8.0 cm bs	0.20	6.50	6.70	10.3	137.3	147.5	8.31
$LSD_{(0.05)}$	1.57	2.12	2.58	56.4	76.5	86.7	1.90
Contrasts							
Non-inoc vs. inoc‡	1.92**	4.37**	6.29**	61.2**	95.3**	156.4**	1.82*
Liq vs. peat	2.57**	1.04	3.60**	106.6**	22.9	129.5**	1.76*
Liq A vs. liq B	1.90*	1.60	3.50**	42.8	22.2	65.0	1.39
Peat A vs. peat B	1.63*	0.57	2.20	21.0	22.0	10.0	0.91
Liq+peat vs. gran	1.36**	4.04**	2.68**	64.3**	72.3**	8.1	0.63
Gran ws vs. gran bs	1.99**	2.12**	0.13	58.1**	19.2	38.9	0.28
Gran 2.5 vs. gran 8.0	0.75	0.01	0.74	16.1	6.2	10.0	0.50
Gran A vs. gran B	0.21	1.24*	1.03	18.0	10.3	7.7	0.34
Gran str x ws vs. bs	0.33	0.02	0.31	0.4	16.0	16.3	0.37
Gran str x 2.5 vs. 8.0	0.45	0.49	0.04	8.9	2.4	2.4	0.21

*, ** Significant at 0.05 and 0.01 levels, respectively.

[‡] Difference.

g pl = g plant⁻¹

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 22. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at Outlook location, 1998.

	Nodul	e no. plant	-1	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt.
Inoculant †	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0	0.10	0.10	0	3.5	3.5	4.15
Liquid A	0.05	0	0.05	4.0	0	4.0	3.75
Liquid B	2.40	1.35	3.75	88.5	50.0	138.5	4.45
Peat A	4.15	2.75	6.90	217.5	90.0	307.5	4.30
Peat B	4.40	3.35	7.75	130.5	45.5	176.0	4.55
Gran A ws	2.00	6.45	8.45	60.0	169.5	229.5	5.05
Gran A 2.5 cm	0.20	6.20	6.40	3.5	200.5	204.0	5.85
Gran A 8.0 cm	0	5.75	5.75	0	133.0	133.0	6.40
Gran B ws	1.65	4.85	6.50	71.5	113.5	185.0	5.20
Gran B 2.5 cm	0.60	5.45	6.05	13.0	105.5	118.5	5.50
Gran B 8.0 cm	0	4.80	4.80	0	144.0	144.0	5.75
LSD _(0.05)	1.11	2.65	2.86	67.9	70.4	89.6	1.46

[†] Gran = granular, ws = with seed.

Table 23. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at Watrous locations, 1998.

	Nod	ule no. pla	ant ⁻¹	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt.
Inoculant †	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0	0.15	0.15	0	2.5	2.5	5.24
Liquid A	0.30	0.70	1.00	15.0	32.0	47.0	4.57
Liquid B	1.60	1.45	3.05	64.0	46.0	110.0	5.01
Peat A	2.40	2.00	4.40	169.0	51.5	220.5	5.96
Peat B	3.35	1.25	4.60	170.5	36.5	207.0	6.22
Gran A ws	2.85	4.45	7.30	100.5	156.5	257.0	7.47
Gran A 2.5 cm	2.05	6.25	8.30	40.0	164.0	204.0	6.04
Gran A 8.0 cm	0.80	7.65	8.45	15.5	259.5	275.0	7.66
Gran B ws	2.60	4.55	7.15	103.0	155.5	258.5	5.99
Gran B 2.5 cm	1.55	6.75	8.30	38.0	157.5	195.5	6.32
Gran B 8.0 cm	1.20	7.65	6.30	38.0	125.0	163.0	6.91
LSD _(0.05)	1.40	2.46	2.97	76.3	76.2	96.3	2.57

† Gran = granular, ws = with seed.

Table 24. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the late pod-filling stage at Watrous locations, 1998.

	No	dule no.pla	ant ⁻¹	Nodule	dry wt. (n	ng plant ⁻¹)	Shoot dry wt
Inoculant †	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0	0.60	0.60	0	17.5	17.5	5.94
Liquid A	0.50	1.70	2.20	19.0	46.5	65.5	7.17
Liquid B	0.75	0.90	1.65	30.0	22.5	52.5	6.00
Peat A	2.50	1.75	4.25	68.0	40.0	108.0	8.75
Peat B	3.30	1.30	4.60	87.0	19.0	106.0	6.65
Gran A ws	3.00	4.15	7.15	77.5	84.5	162.0	11.09
Gran A 2.5 cm	0.90	6.30	7.20	14.5	111.5	126.0	11.33
Gran A 8.0 cm	0	5.60	5.60	0	92.0	92.0	8.45
Gran B ws	2.15	3.70	5.85	30.5	70.5	101.0	7.65
Gran B 2.5 cm	1.00	4.50	5.50	20.5	81.5	102.0	8.56
Gran B 8.0 cm	0.65	6.10	6.75	7.5	108.5	116.0	9.39
LSD _(0.05)	1.05	1.58	2.12	28.6	41.7	52.1	2.04

Gran = granular, ws = with seed.

As in the desi, seed treatment with liquid inoculants in 1998 resulted in low nodule numbers and dry weight in the kabuli and in particular, liquid A was not statistically different from the non-inoculated control (Table 21). Although the difference between the numbers of nodules for the two liquid formulated inoculants was significant (P = 0.01), that for the nodule dry weight was not significant. Similarly, no significant difference in nodule dry weight was observed between the seed-applied inoculant and the granular inoculant treatments despite the significant (P = 0.00) differences observed between the total numbers of nodules. At the early pod-filling stage in kabuli, the highest nodule dry weight at Watrous occurred in peat B treatment (317.5 mg plant⁻¹) (Table 25) whereas at Outlook the highest was 206 mg plant in peat A treatment (Table 26). Sampling at Watrous at the late pod-filling stage revealed a similar trend in nodulation as at early pod-filling but total nodule numbers and dry weight at this growth stage were greatest in granular B placed with the seed (Table 27). Except for the significant (P =0.02) interaction between location and inoculation treatment for total nodule dry weight in kabuli, no significant interactions were observed for total numbers and dry weight of nodules in both desi and kabuli at early pod-filling (analysis not shown).

Table 25. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage at the Watrous location, 1998.

	Nodule no. plant ⁻¹		Nodule dry wt. (mg plant ⁻¹)			Shoot dry wt.	
Inoculant †	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0.10	0.85	0.95	3.0	30.0	33.0	8.06
Liquid A	0.70	2.15	2.85	38.5	99.0	137.5	9.06
Liquid B	3.35	3.90	7.25	100.5	107.5	208.0	7.72
Peat A	3.65	3.00	6.65	157.5	105.0	262.5	10.65
Peat B	6.00	3.50	9.50	226.5	91.0	317.5	11.27
Gran A ws	3.95	6.65	10.60	91.5	128.0	219.5	9.28
Gran A 2.5 cm	1.60	9.75	11.35	21.0	175.5	196.5	10.30
Gran A 8.0 cm	1.15	8.60	9.75	17.5	183.5	201.0	10.74
Gran B ws	4.30	5.90	10.20	125.0	188.0	313.0	10.15
Gran B 2.5 cm	1.55	7.20	8.75	43.5	185.5	229.0	10.53
Gran B 8.0 cm	0.40	9.05	9.45	20.5	221.5	242.0	11.51
LSD _(0.05)	1.10	3.28	3.57	49.9	69.8	82.2	2.22

[†] Gran = granular, ws = with seed.

Table 26. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage at Outlook location, 1998.

	Nodi	ule no. pla	nt ⁻¹	Nodule	Nodule dry wt. (mg plant ⁻¹)		
Inoculant †	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0	0.15	0.15	0	13.0	13.0	5.01
Liquid A	0.40	0.10	0.50	14.5	2.5	17.0	6.50
Liquid B	1.55	1.55	3.10	38.0	38.5	76.5	5.06
Peat A	2.85	2.35	5.20	130.5	75.5	206.0	6.13
Peat B	3.75	3.00	6.75	103.5	67.5	171.0	7.33
Gran A ws	1.05	3.45	4.50	26.5	102.0	128.5	6.45
Gran A 2.5 cm	0.15	7.00	7.15	11.0	153.5	164.5	7.41
Gran A 8.0 cm	0	7.20	7.20	0	162.5	162.5	6.40
Gran B ws	1.70	4.30	6.00	59.5	113.5	173.0	7.80
Gran B 2.5 cm	1.25	4.80	6.05	27.0	81.5	108.5	7.51
Gran B 8.0 cm	0	3.95	3.95	0	53.0	53.0	5.11
LSD _(0.05)	0.92	2.67	2.73	47.9	46.4	62.5	2.42

Gran = granular, ws = with seed.

Table 27. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the late pod-filling stage at Watrous location, 1998.

	Node	ule no. pla	nt ⁻¹	Nodule dry wt. (mg plant ⁻¹)			Shoot dry wt.
Inoculant †	Crown	Lateral	Total	Crown	Lateral	Total	(g plant ⁻¹)
Non-inoculated	0	2.45	2.45	0	76.0	76.0	10.35
Liquid A	3.30	1.65	4.95	29.0	44.0	73.0	9.83
Liquid B	2.00	3.20	5.20	79.0	89.0	168.0	11.73
Peat A	3.60	1.65	5.25	151.5	52.5	204.0	12.82
Peat B	6.25	3.40	9.65	126.5	68.5	195.0	12.44
Gran A ws	2.80	5.00	7.80	69.5	101.0	170.5	13.99
Gran A 2.5 cm	0.85	7.05	7.90	11.5	138.5	150.0	13.66
Gran A 8.0 cm	0.35	10.00	10.35	14.5	195.5	210.0	14.72
Gran B ws	3.50	7.35	10.85	88.0	157.5	245.5	14.27
Gran B 2.5 cm	1.85	8.05	9.90	34.5	179.0	213.5	13.56
Gran B 8.0 cm	0.25	8.25	8.50	11.0	142.0	153.0	12.75
LSD _(0.05)	2.90	2.51	3.51	45.9	57.8	77.1	2.96

Gran = granular, ws = with seed.

Inoculum placement significantly affected the distribution of nodules on the root system and the distribution was consistent across locations in both chickpea types. The peat and liquid inoculants produced more nodules at the crown region, whereas the soil-applied (granular) inoculants produced more lateral root nodules, especially when the granular inoculum were placed below the seed. For example, in the desi experiments, averaged over locations for the 1997 field season, granular inoculant placed at 2.5 and 8.0 cm below the seed formed 72-97% of the nodules (on nodule dry weight basis) on the lateral roots compared to only 25-36% for the peat and liquid inoculants at flowering and early pod-filling stages (Tables 12 and 13). Similarly, 87-97% of the nodules formed by granular inoculant placed below the seed in kabuli were located on the lateral roots compared to 27-54% for the peat and liquid inoculants at early and late pod-filling stages (Tables 14 and 15).

The position of the nodules in 1998 experiments was similar to that in 1997. For example, based on dry weight, granular inoculants placed below the seed produced 79-96% of their nodules on the lateral roots in both chickpea genotypes at the early pod-filling stage as compared to 21-39% in the peat inoculants (Tables 20 and 21).

There were no marked differences among inoculant strains in both chickpea cultivars in both years indicating that the pattern of nodule formation was primarily due to the depth of inoculant placement.

1.2.2 Dry matter yield

At all sampling dates, averaged over locations, shoot dry matter was significantly affected by inoculation methods except for the flowering stage in desi chickpea, even though a similar pattern was observed. For the 1997 experiments, inoculation generally increased shoot dry matter per plant compared to the control, but dry matter yield increases were higher with the granular inoculants placed below the seed than when placed in the seed row or for peat and liquid inoculants (Tables 12 and 13). Orthogonal contrasts confirmed that shoot dry weight in both desi (Tables 12 and 13) and kabuli (Tables 14 and 15) chickpea were significantly higher for soil inoculation as compared to seed inoculation. Moreover, the differences were detected at the 5% level of probability at the flowering stage (Table 8) and significance increased to 1% level at the early and late pod-filling stages (Tables 11-13).

Shoot dry matter for kabuli plants grown from seeds treated with peat-based inoculants was significantly higher than that for the liquid formulated treatments at the early and late pod-filling stages. A significant difference was also observed between the peat-based A and B inoculants at the early pod-filling stage in the kabuli. The interaction between location and inoculation for shoot dry matter was not significant at the early pod-filling stage in both chickpea types but significant interactions were observed in the desi at flowering and in the kabuli at late pod-filling.

For the individual sites, shoot dry matter at the early pod-filling stage in the kabuli at the Watrous site was not affected significantly by inoculation methods in 1997, although it increased when granular inoculant was placed below the seed and when peat-based inoculant B was used, as compared the control (Table 16). In the same way, differences among inoculant treatments and inoculation methods were not significant at Elbow for desi chickpea at both the flowering and the early pod-filling stages (Tables 10 and 11).

Shoot dry weight data for the 1998 field season indicated that the granular inoculant treatments were significantly better as compared to the seed-applied inoculants for the desi (Table 20) but not for the kabuli chickpea (Table 21). However, contrast analysis indicated that placing the granular inoculant 8 cm below the seed resulted in higher shoot dry weight compared to 2.5-cm below seed placement in the desi at the early pod-filling stage. For both chickpea cultivars, the peat-based inoculants were superior to the liquid formulated inoculant in shoot dry matter. The location by inoculation interaction was not significant for shoot dry matter for both desi and kabuli at the early pod-filling stage as in the 1997 field season indicating that the inoculants performed similarly across locations (analysis not shown).

In general, dry matter yields in 1998 both for desi (Tables 22 and 23) and kabuli (Tables 25 and 26) at Watrous were higher than that at Outlook at the early pod-filling stage. For example, the highest shoot dry matter in the kabuli at Watrous was 48% higher than the highest obtained in the kabuli at Outlook. In 1998, plant dry matter yields at the late pod-filling stage were evaluated only at the Watrous site. For desi, dry matter at this stage for the non-inoculated control was not significantly different from that for the liquid inoculants and peat B (Table 24). Inoculation with granular A in the seed furrow and placement at 2.5 cm below the seed significantly increased shoot dry matter

corresponding to 87 and 91% respectively, over the non-inoculated controls. As in the desi, shoot dry matter in the kabuli was lower in the liquid inoculant treatments than in all the other inoculant treatments (Table 27). Although dry matter yield increases were higher for the granular inoculants compared to the peat-based inoculants, they did not differ statistically.

1.2.3 Biomass and Seed Yield

At final harvest in 1997, plant biomass and seed yield per hectare, averaged over locations were significantly increased by inoculation, and granular inoculant placed below the seed and seed inoculated with peat inoculant A produced the highest yields (Tables 28 and 29). For the kabuli chickpea, seed yield increases over the control were 33% for the liquid inoculants, 58% for the peat-based inoculants, 96% for the granular inoculant A placed below the seed and 67% for granular inoculant B placed below the seed (Table 29). Compared to the inoculants placed below the seed, when the granular inoculants were placed in the seed furrow, kabuli chickpea seed yields were 18 and 13% lower for inoculants A and B, respectively, although the differences were not significant. Similar trends were also observed in the desi chickpea, but the seed yield increase due to inoculation was less than that for the kabuli chickpea. For example, granular inoculant placed below the seed increased seed yield by 48%, whereas seed treatment with peat or liquid inoculants increased seed yield by 41 and 26%, respectively (Table 28).

The differences in plant biomass and seed yield between inoculants placed in the seed furrow and placement below the seed were significant. Desi seed yield at Elbow was not consistent with the other sites because the non-inoculated control yielded more than the liquid A treatment, and liquid C produced greater seed yield than all the other treatments except for granular inoculant B placed below the seed (Table 30). Among the inoculation treatments only granular inoculant B placed 2.5 cm below the seed gave significantly higher seed yield as compared to the non-inoculated control at this site. Similarly, desi seed yield at Outlook increased with inoculation, but the increases were not significant at 5% level, with the exception of peat A and granular A placed 2.5 cm below the seed (Table 31). These inconsistencies resulted in significant interactions between location and inoculant treatment for plant biomass and seed yield in the desi experiments, unlike the kabuli experiments. For both desi and kabuli experiments, biomass and seed yields were higher at Watrous (Tables 32 and 34) than the other sites.

Table 28. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), averaged over Elbow, Kenaston, Outlook and Watrous locations for Myles desi chickpea, 1997.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa
Non-inoc	1757	962	176	32.8
Liq A	2184	1211	187	41.4
Liq C	2188	1218	192	48.3
Peat A	2434	1390	197	58.7
Peat B	2328	1329	201	62.9
Gran A with seed	2199	1237	196	50.8
Gran A 2.5 cm bs	2437	1385	215	60.6
Gran A 8.0 cm bs	2578	1466	201	60.5
Gran B with seed	2113	1171	199	56.2
Gran B 2.5 cm bs	2469	1392	207	62.5
Gran B 8.0 cm bs	2556	1451	205	64.0
LSD _(0.05)	411	246	16	10.0
Orthogonal contrasts				
Non-inoc vs. inoc [‡]	592**	363**	24**	23.8*
Liq vs. peat	195	145	10*	16.0*
Liq A vs. liq C	4	7	5	6.9
Peat A vs. peat B	106	61	4	4.2
Liq+peat vs. gran	109	63	10**	6.3**
Gran ws vs. gran bs	354**	210**	10*	8.4**
Gran 2.5 vs. gran 8.0	114	70	8	0.7
Gran A vs. gran B	25	25	0	3.6
Gran str x ws vs. bs	47	25	4	2.3
Gran str x 2.5 vs. 8	27	11	6	0.8

*, ** Significant at 0.05 and 0.01 levels, respectively.

Difference.

^{*}Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 29. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), averaged over Kenaston and Watrous locations for Sanford kabuli chickpea, 1997.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa
Non-inoc	1563	658	173	30.3
Liq A	1810	821	189	44.9
Liq C	1938	928	190	60.9
Peat A	2376	1159	217	62.6
Peat B	1978	929	203	67.6
Gran A with seed	2094	1054	214	70.6
Gran A 2.5 cm bs	2720	1290	220	70.2
Gran A 8.0 cm bs	2585	1291	219	66.0
Gran B with seed	2068	975	214	64.8
Gran B 2.5 cm bs	2324	1090	212	69.3
Gran B 8.0 cm bs	2326	1105	209	67.0
LSD _(0.05)	541	306	14	13.5
Orthogonal contrasts				
Non-inoc vs. inoc‡	659**	406**	36**	34.1*
Liq vs. peat	303	170	21**	12.2
Liq A vs. liq C	128	107	1	16.04
Peat A vs. peat B	398	230	14*	5.0
Liq+peat vs. gran	327**	175*	15**	9.0**
Gran ws vs. gran bs	408*	180	1	0.4
Gran 2.5 vs. gran8.0	67	8	2	3.3
Gran A vs. gran B	227	155	6	1.9
Gran str x ws vs. bs	53	21	1	4.1
Gran str x 2.5 vs. 8	69	7	1	1.0

*, ** Significant at 0.05 and 0.01 levels, respectively.

Difference.

^{*}Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 30. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), at Elbow for Myles desi chickpea, 1997.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa
Non-inoculated	1195	755	182	28.2
Liquid A	1215	705	176	29.1
Liquid C	1775	1030	196	47.0
Peat A	1643	960	202	48.8
Peat B	1500	965	181	57.9
Gran A with seed	1425	893	198	49.5
Gran A 2.5 cm bs	1430	880	223	51.6
Gran A 8.0 cm bs	1545	943	194	41.9
Gran B with seed	1258	738	191	51.1
Gran B 2.5 cm bs	1980	1173	226	65.5
Gran B 8.0 cm bs	1733	1043	188	61.1
LSD _(0.05)	678	355	33	16.5

Gran = granular, bs = below seed

Table 31. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), at Outlook for Myles desi chickpea, 1997.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa
Non-inoculated	1143	653	187	10.8
Liquid A	1628	875	199	32.8
Liquid C	1310	750	215	14.8
Peat A	1803	1100	213	44.9
Peat B	1260	773	216	48.1
Gran A with seed	1413	843	210	22.6
Gran A 2.5 cm bs	1755	1045	227	40.0
Gran A 8.0 cm bs	1610	923	224	48.6
Gran B with seed	1498	860	212	36.9
Gran B 2.5 cm bs	1498	888	216	38.7
Gran B 8.0 cm bs	1535	915	221	44.0
LSD _(0.05)	ns	373	22	17.6

Gran = granular, bs = below seed.

Table 32. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), at Watrous for Myles desi chickpea, 1997.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfe
Non-inoculated	3200	1468	187	53.2
Liquid (A)	4118	1728	189	60.9
Liquid (C)	3820	1668	189	67.2
Peat (A)	4220	1790	204	74.2
Peat (B)	4305	1875	192	74.9
Gran. (A)-seed	3805	1713	195	60.4
- 2.5 cm	3955	1718	221	78.3
- 8.0 cm	4443	1898	204	76.7
Gran. (B)- seed	3278	1448	212	63.6
- 2.5 cm	4113	1823	209	68.4
- 8.0 cm	3828	1658	220	77.8
LSD _(0.05)	823	323	41	16.2

[†] Gran = granular, ws = with seed.

Table 33. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), at Kenaston for Myles desi chickpea, 1997.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa
Non-inoculated	1490	708	147	38.8
Liquid A	1778	875	182	43.0
Liquid C	1845	940	167	64.2
Peat A	2070	1070	192	66.7
Peat B	2245	1150	193	70.5
Gran A with seed	2155	1120	183	70.5
Gran A 2.5 cm bs	2608	1378	188	72.5
Gran A 8.0 cm bs	2713	1453	183	74.5
Gran B with seed	2418	1258	183	73.2
Gran B 2.5 cm bs	2288	1218	179	77.5
Gran B 8.0 cm bs	3127	1678	191	73.3
LSD _(0.05)	388	216	45	14.7

† Gran = granular, ws = with seed.

Table 34. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), at Watrous for Sanford kabuli chickpea, 1997.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa
Non-inoculated	1823	820	172	23.8
Liquid A	2180	1053	193	36.9
Liquid C	2180	1095	185	51.0
Peat A	2698	1355	216	55.2
Peat B	1913	893	201	50.8
Gran A with seed	2230	1158	212	63.1
Gran A 2.5 cm bs	2598	1275	215	59.5
Gran A 8.0 cm bs	2808	1505	209	56.2
Gran B with seed	1993	985	206	46.3
Gran B 2.5 cm bs	2418	1170	208	52.8
Gran B 8.0 cm bs	2323	1125	199	54.2
LSD _(0.05)	591	316	17	13.3

† Gran = granular, ws = with seed.

In 1998, biomass and seed yields at the Outlook location were affected by drought, and the effect was more severe in the treatments where the inoculants were placed below the seed in the kabuli experiment. With granular inoculation (e.g., granular inoculant B placed 8 cm below the seed), biomass and seed yield in the kabuli declined by 25 and 61%, respectively, as compared to the non-inoculated control (Table 35). Although inoculation increased biomass and seed yield at Watrous, the effect was not significant except for the biomass yield enhancement due to granular A placed with the seed (Table 36). Averaged over locations seed inoculation with peat and granular inoculants placed with the seed in the kabuli resulted in higher yields compared to the other treatments (Table 35). However, a contrast of liquid plus peat-based inoculants versus granular inoculants was not significant for both biomass and seed yields.

Unlike the results from 1997 and the desi experiments in 1998, plant biomass for the kabuli in 1998 was significantly higher (P = 0.03) in granular A than granular B inoculant treatments, although the difference in seed yield was not significant (Table 35). At Outlook (Table 38), desi biomass and seed yields were significantly increased by inoculation but unlike Watrous (Table 39), both biomass and seed yields for granular inoculants placed 8 cm below seed were lower than the other granular inoculant treatments and the peat inoculants.

Desi biomass and seed yield averaged over locations were significantly higher in the inoculation treatments as compared to the control (Table 40). However, as in the 1997 experiments, the peat and granular inoculants were superior to the liquid inoculants. On average inoculating the soil with granular inoculants consistently increased biomass and seed yields in the desi. Despite the apparent differences in the kabuli experiments, no significant location by inoculation interactions were observed for biomass and seed yields in both chickpea types in 1998.

Table 35. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), averaged over Outlook and Watrous locations for Sanford kabuli chickpea, 1998.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa
Non-inoc	3742	1218	190	7.5
Liq A	3734	1251	207	21.1
Liq B	3717	1246	207	28.5
Peat A	3940	1317	239	40.6
Peat B	3958	1393	233	41.7
Gran A with seed	4361	1362	243	40.7
Gran A 2.5 cm bs	4190	1268	246	44.2
Gran A 8.0 cm bs	4062	1176	243	37.3
Gran B with seed	4062	1360	230	44.1
Gran B 2.5 cm bs	3869	1185	243	39.4
Gran B 8.0 cm bs	3487	954	258	30.3
LSD _(0.05)	613	285	42	16.1
Orthogonal contrasts				
Non-inoc vs. inoc‡	196	33	45**	29.3*
Liq vs. peat	224	107	29	16.4*
Liq A vs. liq B	17	5	0	7.4
Peat A vs. peat B	18	76	6	1.1
Liq+peat vs. gran	168	84	22*	6.4
Gran ws vs. gran bs	310	215*	11	4.6
Gran 2.5 vs. gran 8.0	255	162	6	8
Gran A vs. gran B	398*	102	0	2.8
Gran str x ws vs. bs	270	108	0	4.2
Gran str x 2.5 vs. 8	127	70	9	1.1

*, ** Significant at 0.05 and 0.01 levels, respectively.

[‡] Difference.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 36. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), at Watrous for Sanford kabuli chickpea, 1998.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa	
Non-inoculated	5265	1528	201	11.1	
Liquid A	5128	1560	237	35.4	
Liquid B	5278	1563	237	38.4	
Peat A	5708	1733	237	41.7	
Peat B	5585	1711	247	42.7	
Gran A with seed	6433	1800	251	40.2	
Gran A 2.5 cm bs	5675	1624	243	46.3	
Gran A 8.0 cm bs	5823	1654	237	37.7	
Gran B with seed	5648	1715	236	44.7	
Gran B 2.5 cm bs	5685	1617	248	41.9	
Gran B 8.0 cm bs	5318	1552	248	41.5	
$LSD_{(0.05)}$	1079	ns	ns	10.5	

Gran = granular, bs = below seed.

Table 37. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), at Outlook for Sanford kabuli chickpea, 1998.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa	
Non-inoculated	2220	909	179	4.0	
Liquid A	2340	943	178	6.7	
Liquid B	2156	929	177	18.6	
Peat A	2173	901	240	39.4	
Peat B	2332	1075	218	40.7	
Gran A with seed	2289	923	235	41.1	
Gran A 2.5 cm bs	2705	911	249	42.1	
Gran A 8.0 cm bs	2302	754	250	36.9	
Gran B with seed	2476	1005	224	43.5	
Gran B 2.5 cm bs	2054	754	237	36.9	
Gran B 8.0 cm bs	1657	357	268	19.2	
LSD _(0.05)	478	224	22	8.7	

Gran = granular, bs = below seed.

Table 38. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed, at Outlook for Myles desi chickpea, 1998.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa
Non-inoculated	1486	846	143	6.6
Liquid A	2118	1156	153	11.8
Liquid B	2037	1152	145	36.3
Peat A	2639	1478	167	51.9
Peat B	2512	1455	184	49.2
Gran A with seed	2603	1462	185	55.3
Gran A 2.5 cm bs	2733	1504	229	62.8
Gran A 8.0 cm bs	2295	1310	234	57.9
Gran B with seed	2453	1411	193	46.0
Gran B 2.5 cm bs	2349	1412	209	54.9
Gran B 8.0 cm bs	2065	1210	203	50.9
LSD _(0.05)	551	318	26	14.2

Gran = granular, bs = below seed.

Table 39. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), at Watrous for Myles desi chickpea, 1998.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa	
Non-inoculated	3204	1598	170	27.7	
Liquid A	3993	1886	184	27.9	
Liquid B	3906	1839	202	51.7	
Peat A	4369	2147	219	46.4	
Peat B)	4615	2048	249	58.1	
Gran A with seed	4863	2148	215	58.4	
Gran A 2.5 cm bs	4410	1993	245	57.8	
Gran A 8.0 cm bs	4557	2070	226	60.6	
Gran B with seed	4552	2099	222	63.8	
Gran B 2.5 cm bs	4569	2051	234	54.3	
Gran B 8.0 cm bs	4770	2242	250	56.1	
LSD _(0.05)	575	282	25	12.0	

Gran = granular, bs = below seed.

Table 40. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), averaged over Outlook and Watrous locations for Myles desi chickpea, 1998.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa
Non-inoc	2311	1222	156	17.2
Liq A	3056	1521	169	19.9
Liq B	2971	1495	174	44.0
Peat A	3504	1813	193	49.1
Peat B	3564	1751	217	53.7
Gran A with seed	3733	1805	200	56.9
Gran A 2.5 cm bs	3571	1748	237	60.3
Gran A 8.0 cm bs	3418	1690	230	59.3
Gran B with seed	3503	1755	208	54.9
Gran B 2.5 cm bs	3459	1731	222	54.6
Gran B 8.0 cm bs	3418	1726	226	53.5
LSD _(0.05)	490	210	32	14.7
Orthogonal contrasts				
Non-inoc vs. inoc‡	1109**	482**	52**	33.4*
Liq vs. peat	521**	274**	34**	19.5*
Liq A vs. liq B	85	26	5	24.1*
Peat A vs. peat B	60	62	24	4.6
Liq+peat vs. gran	243*	98*	32**	14.94
Gran ws vs. gran bs	152	56	25*	1.0
Gran 2.5 vs. gran 8.0	97	32	2	1.1
Gran A vs. gran B	114	10	4	4.5
Gran str x ws vs. bs	141	38	2	1.4
Gran str x 2.5 vs. 8	56	27	6	0.1

*, ** Significant at 0.05 and 0.01 levels, respectively.

[‡] Difference.

1.2.4 Seed Protein Concentration and Nitrogen Fixation

Seed protein and %Ndfa for the seed averaged over locations in 1997 were significantly higher for inoculated plants in both cultivars, and the highest protein concentration and %Ndfa generally occurred when soil inoculation was used, particularly when granular inoculant A was placed 2.5 cm below the seed (Tables 28 and 29). In both kabuli and desi, seed inoculated with liquid formulated inoculants resulted in lower N₂ fixation and seed protein concentration than all the other inoculation treatments. However, except for liquid A, the N₂ fixation associated with liquid C was not statistically different from all the other inoculation treatments in the kabuli. In contrast to

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

the general trend, seed inoculation with peat A produced significantly (P = 0.05) higher seed protein concentration than that for peat B in the kabuli. For desi, placing the granular inoculants below the seed significantly increased N_2 fixation (P = 0.01) and seed protein concentration (P = 0.04) as compared to placement in the seed furrow. As was observed for the other yield parameters, no differences associated with *Rhizobium* strain was observed for both chickpea genotypes.

The highest N_2 fixation and protein concentration in the 1997 kabuli experiments were 85.8% and 230 g kg⁻¹ seed, respectively, and occurred at Kenaston (Table 41). In the kabuli experiments, N_2 fixation and seed protein concentration were greater at Kenaston than Watrous (Table 36) but significant (P = 0.03) location x inoculation interactions were detected for N_2 fixation. For the desi experiments, N_2 fixation was generally higher at Kenaston (Table 33) and Watrous (Table 32), whereas seed protein concentration was higher at Outlook (Table 3). There were no significant location x inoculation interactions for protein concentration and %Ndfa in desi.

Table 41. Biomass, seed yield, protein concentration and percentage N derived from atmosphere for the seed (%Ndfa), at Kenaston for Sanford kabuli chickpea, 1997.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Protein content (g kg ⁻¹)	%Ndfa	
Non-inoculated	1303	495	174	36.7	
Liquid A	1440	590	184	52.9	
Liquid C	1695	760	195	70.8	
Peat A	2055	963	218	69.9	
Peat B	2043	965	206	84.5	
Gran. A with seed	1958	950	216	78.0	
Gran A 2.5 cm bs	2843	1305	226	80.9	
Gran A 8.0 cm bs	2363	1077	230	75.8	
Gran B with seed	2143	965	222	83.4	
Gran B 2.5 cm bs	2230	1010	215	85.8	
Gran B 8.0 cm bs	2330	1085	218	79.7	
LSD _(0.05)	497	282	17	10.1	

Gran = granular, ws = with seed.

Nitrogen fixation and seed protein concentration in 1998 for both desi (Table 40) and kabuli (Table 35) chickpea followed a similar trend as observed in the 1997 experiments. However, although the average levels of N fixed were lower in 1998, the average seed protein concentration was higher in 1998 than 1997. In general, granular inoculants placed below the seed in both cultivars resulted in the highest levels of N₂ fixation and the highest seed protein concentrations. As with the other parameters measured, the liquid inoculants were inferior to the other inoculation treatments in terms of N₂ fixation and seed protein concentration. In contrast to the 1997 experiments, significant interactions between location and inoculation were detected for N₂ fixation and seed protein concentration in both kabuli and desi.

1.2.5 Relative Contribution of Crown and Lateral Root Nodules

The relationship between shoot dry matter and the dry weight of the corresponding crown or lateral root nodules on an individual plant basis for kabuli and desi are illustrated in Figs. 1 and 2, respectively. The results from 1997 shown in Figs. 1 and 2 are averaged over the two or the four experimental sites respectively, for the kabuli and desi experiments. Regression analyses revealed a significant positive correlation between shoot dry matter and dry weight of lateral root nodules at all sampling dates in both kabuli and desi chickpeas. In contrast, the relationship between shoot dry matter and dry weight of crown nodules was poor and negative. For example, in the kabuli at the early pod-filling, the coefficients of determination (r^2) for the regression equation was 0.84 (P = 0.000) for shoot dry matter against dry weight of lateral root nodules whereas against the dry weight of crown nodules, the r^2 was 0.05 (P = 0.52) (Fig. 1). A similar trend was observed in the desi at the early pod-filling stage (Fig. 2). As the kabuli plants approached maturity, the relationship between the shoot dry matter and dry weight of lateral root nodules was not as strong ($r^2 = 0.67$) as that at the early pod-filling stage, although it remained highly significant (P = 0.002).

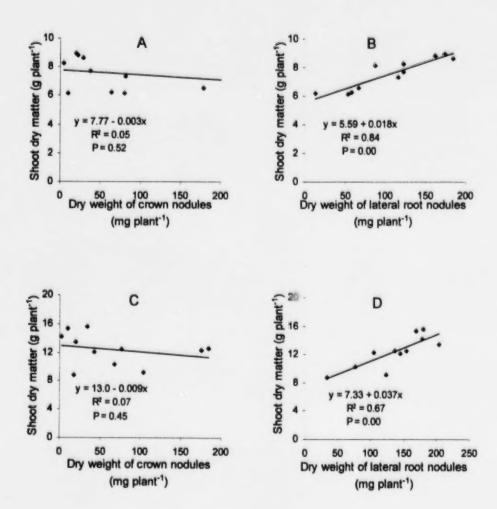
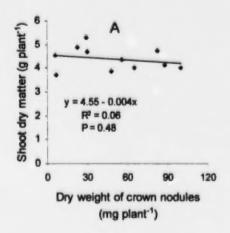


Figure 1. Relationship between shoot dry matter per plant and crown or lateral root nodules per plant at the early (A and B) and late pod-filling (C and D) stages in the kabuli in 1997.



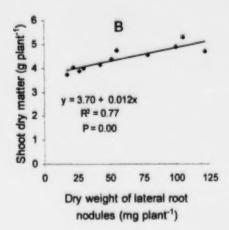


Figure 2. Relationship between shoot dry matter per plant and crown (A) or lateral (B) root nodules per plant at the early pod-filling stage in the desi in 1997.

Similarly, regressions of seed yield separately on dry weight of lateral root nodules and crown nodules showed that the lateral root nodules correlated well with seed yield although the relationship was not as strong as that observed for shoot dry matter (Figs. 3.3 and 3.4). For example, for the lateral root nodules at the early pod-filling stage, the regression coefficients were 0.54 (P = 0.01) and 0.48 (P = 0.02) in the kabuli and desi experiments, respectively. For the crown nodules, the regression coefficients were 0.01 (P = 0.79) and 0.00 (P = 0.96) in the kabuli and desi experiments, respectively. Unlike the association between shoot dry matter and nodule dry weight, which deteriorated as the plant approached maturity, the association between seed yield and nodule dry weight at late pod filling in the kabuli improved (e.g., $r^2 = 0.64$, P = 0.003 for lateral root nodules and $r^2 = 0.01$, P = 0.72 for crown nodules).

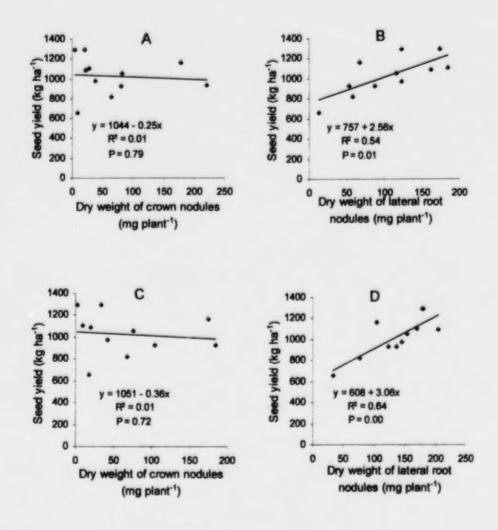


Figure 3. Relationship between seed yield and crown or lateral root nodules per plant at the early (A and B) and late pod-filling stages (C and D) in the kabuli in 1997.

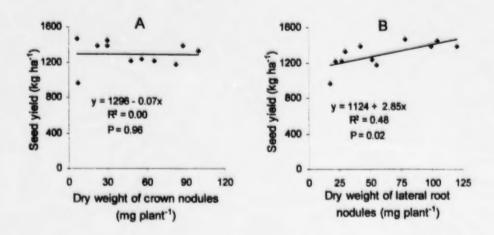


Figure 4. Relationship between seed yield and crown (A) or lateral (B) root nodules per plant at the early pod-filling stage in the desi in 1997.

Similarly, in 1998 a significant linear relationship existed between shoot dry matter and the dry weight of lateral root nodules at early pod-filling but no significant correlation was found between shoot dry matter and dry weight of crown nodules in both cultivars (Fig 3.5). However, unlike 1997 where the relationship was stronger in kabuli, the regression coefficients for 1998 indicate that the lateral root nodules correlated more precisely with shoot dry matter in the desi ($r^2 = 0.82$, P = 0.000) than the kabuli ($r^2 = 0.43$, P = 0.03) at the early pod-filling stage. This observation is likely related to the late germination resulting from drought conditions in the kabuli at Outlook.

In 1998, seed yield was not related to dry weight of lateral root nodules at early pod-filling in the kabuli although it correlated with dry weight of crown nodules ($r^2 = 0.44$, P = 0.03) (Fig. 6). In contrast, the seed yield correlated significantly with dry weight of lateral root nodules at early pod-filling in the desi but the relationship with dry weight of crown nodules was not significant.

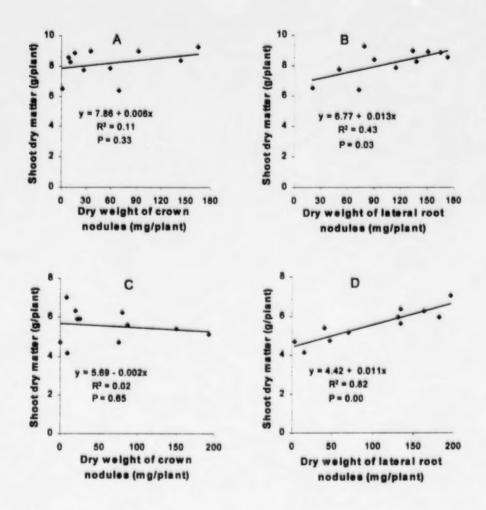


Figure 5. Relationships between shoot dry matter per plant and crown (A) or lateral (B) root nodules per plant in kabuli, and crown (C) or lateral (D) root nodules per plant in desi at early pod-filling stage in 1998.

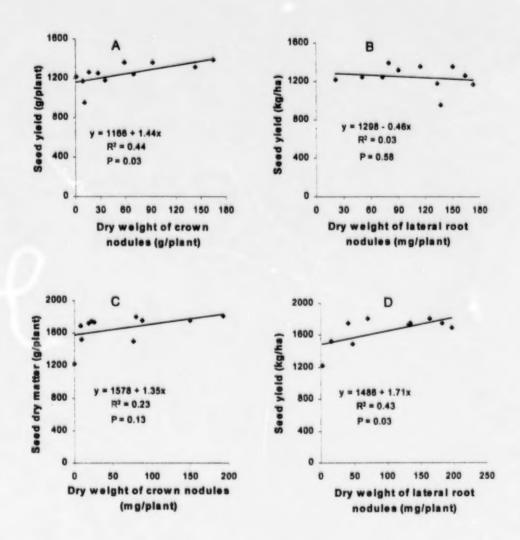


Figure 6. Relationships between seed yield and crown (A) or lateral (B) root nodules per plant in kabuli, and crown (C) or lateral (D) root nodules per plant in desi at early pod-filling stage in 1998.

1.3 Discussion of the Effect of Inoculant Formulation and Placement on Biological N₂ Fixation by Chickpea

Separate experiments were conducted for both desi and kabuli chickpea. Although not consistent in all instances, the two genotypes responded similarly to method of inoculation and rhizobial strain combinations. Somasegaran et al. (1988) reported that under certain soil conditions, and with the use of mixed inoculant strains, a significant strain x genotype interaction can occur. The inoculant obtained from MicroBio RhizoGen Corp. designated A and Agrium Biologicals, designated C, consisted of single strains whereas that obtained from LiphaTec, designated B, was a mixture of three strains. However, strains in similar formulations performed equally throughout the experiments and there were no evidence to indicate differential response to rhizobial strains within or between genotypes in any of the parameters measured.

With the exception of Elbow in 1997 and Outlook in 1998, seeding conditions (i.e., soil moisture) was good to excellent and generally favoured the establishment of a successful symbiosis. Nevertheless, there was evidence that a decline in rhizobia numbers might have occurred in the liquid inoculant treatments either prior to seeding or in the seedbed in that nodulation was generally lower than that for the peat and granular inoculants. For example, the extremely poor nodulation in the liquid treatments at Outlook in 1998 was exacerbated by severe drought during and after seeding. On the other hand the soil moisture status at Watrous in 1997 was relatively good; hence nodulation in the liquid inoculant treatment was comparable to that of the other treatments.

Peat as inoculant carrier protects rhizobia inoculated onto seed to some extent from desiccation (Hansen, 1994); hence, rhizobia in liquid-based inoculant inoculated onto seed are relatively more susceptible to unfavorable environmental conditions such as desiccation and excessive heat during and after seeding. Roughley (1993) demonstrated the effect of desiccation on rhizobia inoculated onto seed where 95% of the rhizobia originally present in the inoculant died during inoculation and sowing, and further 85% of the remaining rhizobia lost viability during the following day in the soil. Death of rhizobia on seed between inoculation and seeding due to high temperature was also reported by Brockwell et al. (1987).

Nodule numbers in the peat and granular inoculant treatments were not consistent over the years. In 1997 the peat inoculants produced more nodules than the granular inoculants but in 1998 the granular inoculants formed more nodules than the peat inoculants. However, no consistent relationship between total nodule dry weight and total nodule numbers existed. Despite the higher total nodule numbers in the peat inoculant treatments in 1997, the total dry weights were similar to that for the granular inoculants. This agrees with previous observations by Smith et al. (1981) and Danso and Bowen (1989) indicating that when soybeans have only few nodules on their roots they tend to grow much larger than on plants that have many nodules.

In contrast to the observation in 1997, the dry weights of the nodules for the peatbased inoculants in 1998 were generally higher than that for the granular inoculants. This observation was, however, more pronounced at Outlook than at Watrous. Planting at Outlook in 1998 was on 20 May, when the soil was dry (mean monthly precipitation was 57% less than normal). As a result of the hard soil surface, the seeder tended to ride up when the sideband disk was set for deeper depths, resulting in the shallow placement of the seed. As a consequence, the seeds were placed in a relatively dry seedbed and germination in these treatments (i.e., where granular inoculants were placed below the seed) occurred well after seeding, following the first significant precipitation event. Hence at the time of sampling, both nodule formation and development in these treatments were behind as compared to other treatments. This artifact of seeding may account for the lower dry weight in the treatments where granular inoculants were placed at depth.

In light of the delayed germination and growth of the chickpea seedlings, subsequent nodulation by the rhizobia applied as inoculant suggests that the strains survived well, in spite of the drought condition. This is consistent with the report by Brockwell et al. (1980), which stated that when conditions are unfavorable for rhizobial survival, or when germination is delayed due to adverse environmental conditions, soil inoculation produced better nodulation than seed-applied inoculation. In our experiments, the kabuli was more affected by the drought than the desi because it has larger seed and requires relatively more moisture for germination. Therefore nodulation in the kabuli was lower at Outlook than Watrous in 1998 and, for this reason, a significant site x inoculation interaction was observed for total nodule numbers and dry weight.

Surprisingly, although it is generally assumed that Saskatchewan soils do not contain indigenous rhizobia capable of nodulation chickpea, some nodulation (although very limited) was observed in the uninoculated control treatments. It is possible that this unexpected nodulation was due to low levels of plot-to-plot contamination, despite efforts to minimize this possibility. Growth chamber studies prior to seeding indicated that the soil from all the experimental sites did not contain any native chickpea rhizobia.

In agreement with other workers (Smith et al., 1981; Danso and Bowen, 1989; Kahn and Stoffella, 1991), the dry weight of nodules was considered a more accurate measure of N_2 -fixing potential than nodule numbers due to the wide variation in nodule size. The location of nodule formation on the root system varied depending on the inoculation method. When granular inoculants were used, most of the nodules were formed on the lateral roots, whereas seed inoculation produced nodules predominantly at the crown region of the root. Soil inoculation in the seedrow and below the seed further indicated that the position of nodules also depended on the depth of inoculant placement. The nodulation pattern observed in this study is consistent with data from other studies in which deeper placement of rhizobial inoculants in the soil resulted in substantial nodule formation below the topmost 10 cm region of the root (Wilson, 1975; Wadidirisuk et al., 1989).

The predominant nodulation in the root zone immediately below the position of inoculum placements indicates the limited migration of inoculant strains. According to Madsen and Alexander (1982), a strain of *B. japonicum* could not move more than 2.7 cm in the absence of infiltrating water. Hence, to achieve optimal distribution of nodules on the entire root system, the roots must encounter rhizobia distributed throughout the soil. Moreover, relatively large rhizobial populations may be required (Zablotowicz et al., 1991).

Although not consistently significant, trends suggested that granular inoculants promoted higher shoot dry matter as compared to the peat and liquid inoculants. In addition, the liquid inoculants were typically inferior to the peat inoculants in terms of promoting shoot dry matter production.

Inoculation had little effect on shoot dry matter at both flowering and early pod filling stage in desi at Elbow in 1997. The soil moisture content at Elbow was low as compared to the other sites and could have partly suppressed the full effects of the individual inoculant treatments. Sprent (1972) and Durand et al. (1987) demonstrated the importance of available soil moisture for maximum N₂ fixation by grain legumes.

The value of inoculation (irrespective of formulation) was demonstrated in seed yield and plant biomass at final harvest. Although the yield increases in 1997 were not consistently significant among inoculation treatments and across locations in both chickpea genotypes, there was clear evidence that granular inoculants placed below the seed were superior to the other inoculant treatments. For example, in 1997 the maximum increase in seed yield averaged over locations for kabuli was 633 kg ha⁻¹ and occurred in response to application of granular inoculant A placed below the seed. This yield increase was followed by that associated with granular inoculant B, placed below seed (440 kg ha⁻¹). Seed yield differences between the peat and granular inoculations were relatively small and, in some instances, not statistically significant. For example for kabuli, seed yield increases for granular A and B placed below the seed were 247 kg ha⁻¹ and 54 kg ha⁻¹ respectively, over that achieved when the corresponding peat inoculants were used. For desi, seed yield increases associated with the granular inoculants placed below the seed were 64 kg ha⁻¹ and 209 kg ha⁻¹ over that for the peat and liquid inoculants, respectively.

The higher yields associated with the use of granular inoculants placed below the seed may be due, in part, to the occurrence of more favourable soil moisture and temperature conditions in this soil zone. Thus, placement of inoculants granules below the seed row may have protected the rhizobia and subsequently promoted nodule formation and N₂ fixation. In other studies, yield increases of 38% (Scudder, 1975), 60% (Bezdicek et al., 1978) and 20% (Muldoon et al., 1980) were reported in soybean for granular inoculant over seed-applied inoculant. Dean and Clark (1977) also reported an increase of 730 kg ha⁻¹ seed yield over seed-applied inoculant when granular inoculant was used in a study with faba bean.

Higher seed protein concentration and N derived from the atmosphere for the seed were generally obtained when granular inoculants were used and reflected the trend observed for plant biomass and seed yields. This observation supports earlier reports that soil inoculation produced higher N₂ fixation (Muldoon et al., 1980; Dubetz, et al., 1983; Hardarson et al., 1989) and seed protein concentration (Muldoon et al., 1980) as compared to seed inoculation in other pulse crops.

In 1997, seed inoculation with peat inoculant B resulted in the fixation of considerably higher proportion of N, but this was not translated into higher seed protein concentration. A similar trend was observed in 1998 but to a lesser degree. In general, N₂ fixation was higher in 1997 (maximum 86 and 78% for kabuli and desi, respectively) than 1998 (maximum 46 and 64% for kabuli and desi, respectively) whereas protein

concentration was higher in 1998 than 1997. This supports the negative correlation between yield and protein content, which often occurs in grain legumes under "normal" growing conditions (Williams and Nakkoul, 1983).

The results of the present study indicate that differences in yield parameters were likely influenced by the nodulation pattern rather than the number or dry weight of nodules. Others have similarly concluded that nodulation of lateral roots plays a critical role in determining the N₂ fixation potential of pulses (Hardarson et al., 1989; McDermott and Graham, 1989; Wolyn et al., 1989; Vikman and Vessey, 1992, 1993). Nodules at the crown region are the first to be formed and are active during the early growth stages of plant but according to Bergensen (1958), the activity of such nodules in soybean persists for an average duration of 65 days. Hence nodules which develop later on the lateral roots may be essential since they remain active during the entire period of high N demand at pod-filling and seed maturation (Ciafardini and Barbieri, 1987; Zapata et al., 1987; Imsande, 1989).

Danso et al. (1990), using the ¹⁵N isotope dilution technique, demonstrated that seed inoculation which formed mostly crown nodules, fixed more N₂ than soil inoculation when estimates of fixation were undertaken at relatively early growth stages. This trend, however, was reversed when N₂ fixation was evaluated at physiological maturity. Similarly, McDermott and Graham (1989) found that crown nodules accounted for 100% of the acetylene reduction activity 20 DAP but the activity declined to less than 20%, 76 DAP (pod-fill) due to nodule senescence and the steady increase in nodule mass on the lateral roots. Therefore, granular inoculant directed to the soil will permit the young lateral roots to come into direct contact with the inoculant strain for infection and nodule formation. Thus, granular inoculant enhances lateral root nodulation, which can contribute significantly to N₂ fixation and yield.

Shoot dry matter was regressed separately on dry weight of crown and lateral root nodules on an individual plant basis over all locations. Results from 1997 demonstrated that the dry weight of the lateral root nodules accounted for 67-84% of the variation in shoot dry matter at the early and late pod-filling stages (Figs. 1 and 2). In contrast, the dry weight of the crown root nodules explained only 5-7 % of the variation in shoot dry matter on an individual plant basis (Figs. 3 and 4). Furthermore, dry weight of the lateral nodules on an individual plant basis accounted for 48-64% of the variation in seed yield on a plot basis. Dry weight of the crown nodules on an individual plant basis accounted for only 1% or less of the variation in seed yield on a plot basis. Drought at Outlook during seeding in 1998 diminished the correlation between lateral root nodules and crop yield for kabuli but for the desi, lateral root nodules accounted for a significant amount of variability in shoot dry matter at early pod-filling (82%) (Fig. 5) and seed yield (43%) (Fig. 6). On the other hand, the crown nodules explained only 2% of the variation in shoot dry matter at early pod filling stage and 23% variability in seed yield. The strong association between yield (shoot dry matter and seed) and lateral root nodules indicates that these nodules often determine, to a large extent, the yields of nodulated legumes and thus seem to be have a greater effect on yield than crown nodules.

The data collected throughout this study highlights the potential for improving pulse crop yields by promoting sufficient nodulation of the lateral roots. Due to the limited migration of rhizobia in the soil, seed inoculation often results in crown root

nodulation. As the crown root nodules approach senescence, the plant may be dependent on nodules formed by indigenous strains that may be less efficient in N₂ fixation (Vance and Graham, 1995). Soil inoculation using granular inoculants is one possible way to enhance lateral root nodulation since the inoculum can be positioned in the soil zone to target the young developing roots.

2. Evaluation of Inoculant Formulation for Dryland Bean, and Irrigated Bean and Field Pea Production

The efficacy of granular soil-implant inoculants for dryland bean, and irrigated bean and pea also was evaluated during the 1997 and 1998 field seasons. The irrigated trials were conducted in both 1997 and 1998 in collaboration with Mr. Terry Hogg at the Saskatchewan Irrigation Development Centre (S.I.D.C.), Outlook, SK. The dryland bean trials were conducted at Rosthern and Floral in 1998. Inoculants used in the experiments were prepared by MicroBio RhizoGen Corp. (MBR) and Liphatech Inc..

2.1 Materials and Methods

2.1.1 Irrigated Pea and Bean Field Trials - 1997

In 1997, two experiments were established at the Outlook Irrigation Centre, located at NW 12-29-09-W3, to evaluate the efficacy of granular soil-implant inoculants as compared to peat-based and liquid inoculants applied directly to the seed coat of bean and field pea. MicroBio RhizoGen Corp. supplied both the bean and pea inoculants, formulated either as a liquid, peat-based, or granular inoculants. These "same strain" inoculants were compared with commercially available liquid, peat-based and granular inoculants (Liphatech Inc.).

The experiments were conducted using a randomized complete block design consisting of ten treatments and replicated four times. The treatments were as follows: liquid (brand A and B); peat-based (brand A and B); granular (brand A and B); an uninoculated control; and a flax (var. McGregor) control. In addition, for pea, a granular and peat-based pseudomonad inoculant in combination with granular inoculant (brand A) seed-placed treatments were included. The granular inoculants were placed either in the seed row or beside (2.5 cm) and below (2.5 cm) the seed row. Inoculants with the same designation, e.g., A or B, indicate that identical Rhizobium strain or strains were used in the different carriers. Inoculants designated A contained a single strain (MicroBio RhizoGen, Saskatoon, SK) whereas inoculants designated B contained a mixture of strains (LiphaTec Inc., Milwaukee, W.I). All treatments were fertilized with P₂O₅, supplied as monoammonium phosphate (12-51-0), at a rate of 50 kg ha⁻¹ as a side-band treatment. Bean seed (var. Othello) was treated with a mixture of Captan, Methoxychlor and streptomycin sulfate (a standard treatment in 1997, applied to bean seed purchased through the U.S.) and was seeded at a rate of 30 seeds m⁻². Field pea (var. Carneval) was seeded at a rate of 80 seeds m⁻².

Bean treatment plots consisted of four rows at 60 cm spacing and measured 2.4 m by 6 m. Pea treatment plots consisted of 12 rows at 20 cm spacing and measured 2.4 m by 6 m. Individual treatments were spaced on 3 m centers.

The sites were irrigated as required. The soil, developed on sandy glacial-fluvial and lacustrine deposits, is classified as a Bradwell sandy loam/ loamy fine sand. Although initial spring soil test results indicated low levels of both N and P, soil samples collected from the test areas immediately prior to plot establishment unfortunately revealed relatively high nutrient levels (Table 42). The relatively high levels of available

spring NO₃-N were not anticipated and likely occurred as a consequence of relatively high levels of mineralization prior to seeding.

Table 42. Spring soil analysis for the pea and dry bean inoculant formulation evaluation trial conducted at the Saskatchewan Irrigation Development Centre.

Crop Depth (cm)	Depth	pH	E.C.	NO ₃ -N	P	K	SO ₄ -S
		(1:2)	kg ha ⁻¹				
Pea	0-30	7.8	0.1	31	16	708	9
	30-60	8.1	0.2	36			9
Dry Bean	0-30	7.8	0.2	45	19	815	10
	30-60	8.1	0.2	40			9

Nodule development (size and numbers) on pea roots was evaluated at the flat pod stage, using a nodule rating system developed by Drs. G. Clayton and W. Rice. (pers. comm.). Similarly, nodule development on the bean roots was evaluated at the early pod stage. Plant height was determined by measuring stem length of 5 plants in each individual treatment at the pod fill growth stage. A midseason biomass harvest was conducted for the bean experiment. A similar harvest was not conducted for the field pea experiment because it was determined that the harvest procedure would be too destructive in the dense crop canopy. Final harvest of bean material was conducted by hand (1 m row) for harvest index determinations and for tissue sampling for subsequent ¹⁵N analysis. In addition, both field peas and beans were mechanically harvested using a small-plot combine. For pea, the entire treatment area was cut whereas for bean only the centre two rows were cut from each treatment. The seed samples were cleaned, weighed and a subsample was used to determine seed weight. Biological N₂ fixation determinations were conducted using the ¹⁵N natural abundance technique. Flax (var. McGregor) served as the non-fixing reference crop.

2.2 Results of the Irrigated Bean and Field Pea Trials

2.2.1 Irrigated Bean Field Trials - 1997

There was no significant effect of inoculant, as compared to the control, on plant height, midseason biomass, final biomass, nodule rating, seed yield or harvest index of dry bean in 1997 (Table 43). However, seed N concentration and seed N accumulation was enhanced by the side band application of granular B (Table 44). Similarly, seed N accumulation was enhanced by the application of the granular A, applied in the seed furrow and in a sideband, and by the application of peat B applied to the seed coat. Seed weight was enhanced by both the granular A (sideband and seed-placed) and granular B (sideband) although larger seed size did not translate into higher yields.

Table 43. Effect of inoculant formulation on plant height, plant biomass, nodule rating, seed yield and harvest index of dry bean at the Outlook Irrigation Centre, 1997.

Treatment		Plant Height	Mid- season	Final Biomass	Nodule Rating	Seed Yield	Harvest Index
	Formulation	cm	Biomass kg ha ⁻¹	kg ha ⁻¹		kg ha-1	
A	Liquid	51.8	1523	8000	6.8	2805	0.35
В	Liquid	48.8	1289	7992	7.3	2973	0.37
A	Peat	47.5	1513	8679	6.5	3024	0.35
В	Peat	48.5	1299	8496	6.5	3354	0.40
A	Gran. SP	52.5	1500	9046	6.0	3285	0.38
A	Gran SB	52.5	1289	9167	6.5	3286	0.36
В	Gran SP	48.8	1198	7738	6.3	3004	0.40
В	Gran SB	49.5	1425	9313	4.5	3102	0.36
Uninoc.	Control	50.5	1483	8771	6.0	2888	0.33
	LSD _(0.05)	n.s.	236	n.s.	1.3	474	n.s.

SP = seed-placed

SB = side-band

Table 44. Effect of inoculant formulation on seed N concentration and accumulation and seed weight of dry bean at the Outlook Irrigation Centre, 1997.

Treatment	Formulation	Seed N %	Seed N Uptake kg ha ⁻¹	Seed Weight mg
A	Liquid	2.54	71.7	340
В	Liquid	2.56	76.2	339
A	Peat	2.68	81.1	335
В	Peat	2.74	91.6	341
A	Gran. SP	2.90	95.0	348
A	Gran SB	2.77	90.3	353
В	Gran SP	2.89	86.9	337
В	Gran SB	2.99	92.8	348
Uninoc.	Control	2.56	74.2	330
	LSD _(0.05)	0.34	14.2	13.4

SP = seed-placed

SB = side-band

Estimates of N₂ fixation by beans were highly variable (Table 45). Moreover, estimates obtained using the natural abundance technique and the difference method were not similar in terms of magnitude or relative ranking. The reason for the high degree of variability is not known; however, it is likely that levels of N₂ fixation were, in general, relatively low as a consequence of the relatively high soil N levels.

Table 45. Effect of inoculant formulation on estimates of nitrogen fixation (Ndfa) by dry bean, determined using the Natural Abundance Method and the N Difference Method at the Outlook Irrigation Centre, 1997.

			dfa Abundance	Ndfa N Difference Method		
Treatment	Formulation	%	% kg ha ⁻¹ % 5.0 40.0 20.0 7.4 36.4 25.6 3.7 35.8 30.6 2.4 38.1 38.1 3.3 55.6 41.1	kg ha ⁻¹		
A	Liquid	55.0		20.0	16.1	
В	Liquid	47.4	36.4	25.6	20.6	
Α	Peat	43.7	35.8	30.6	25.5	
В	Peat	42.4	38.1	38.1	36.0	
A	Gran. SP	59.3	55.6	41.1	39.4	
A	Gran SB	60.7			34.7	
В	Gran SP	52.4	44.6	34.2	31.3	
В	Gran SB	68.2	62.8	39.5	37.2	
Uninoc.	Control	52.6	39.6	24.0	18.6	
	LSD _(0.05)	16.8	15.4	11.6	14.2	

SP = seed-placed

SB = side-band

Visual assessments of the treatments indicated that there was no effect of inoculant formulation on days to 50% flowering or maturity. All treatments flowered 51 days after seeding and reached maturity 97 days after seeding.

2.2.2 Irrigated Field Pea Trials - 1997

Although plant height of pea was enhanced by the application of granular inoculants (i.e., both A and B) applied in a sideband, seed weight and seed yield, as well as seed N concentration and accumulation were not significantly enhanced by inoculation with *Rhizobium*, as compared to the control (Table 46).

Similarly, although several of the inoculants enhanced nodule rating as compared to the control treatment, N₂ fixation was not significantly enhanced by the *Rhizobium* inoculants (Table 47). As was observed for the dry beans, estimates of N₂ fixation were variable. Moreover, estimates obtained using the natural abundance technique and the difference method were not similar in terms of relative ranking. The reason for the high degree of variability is not known; however, it is likely that levels of N₂ fixation were, in general, relatively low as a consequence of the relatively high soil N levels.

As was observed for dry beans, there was no effect of inoculants on days to 10% flowering or maturity. All treatments flowered 56 days after seeding and reached maturity 98 days after seeding.

Table 46. Effect of inoculant formulation on plant height, seed weight, seed yield and seed N concentration and uptake in pea at the Outlook Irrigation Centre, 1997.

Treatment	Formulation	Plant Height	Seed Weight	Seed Yield	Seed N	Seed N Uptake
		cm	mg	kg ha ⁻¹	%	kg ha
A	Liquid	95.2	244	4886	3.4	166
В	Liquid	95.5	241	4970	2.94	146
A	Peat	92.5	242	4932	2.88	142
В	Peat	93.5	243	4890	3.10	152
A	Gran. SP	95.2	240	4858	2.59	126
A	Gran SB	96.8	239	4986	3.32	166
В	Gran SP	95.8	241	4874	3.16	154
В	Gran SB	98.2	248	4992	2.92	146
A	Gran. SP +Pseud. Gran.	94.2	239	4756	2.82	134
A	Gran SB + Pseud.	90.8	246	4896	2.32	114
Uninoc	Control	91.5	243	4922	3.07	151
	LSD _(0.05)	5.2	6.0	n.s.	0.53	28

SP = seed-placed, SB = side-band

Table 47. Effect of inoculant formulation on nodule rating and estimates of nitrogen fixation (Ndfa) by field pea, determined using the Natural Abundance Method and the N Difference Method at the Outlook Irrigation Centre, 1997.

Treatment Formulation		Nodule	Ndfa	N	dfa
		Rating Natural Abundance		Difference Method	
			%	%	kg ha'
A	Liquid	5.8	60.9	65.1	109
В	Liquid	5.5	50.6	60.2	89
A	Peat	4.0	49.1	59.0	85
В	Peat	5.5	42.1	61.0	94
A	Gran. SP	5.0	58.4	53.2	69
A	Gran SB	5.5	51.0	65.3	108
В	Gran SP	3.8	56.8	61.7	97
В	Gran SB	5.2	57.5	60.4	88
A	Gran. SP + Pseud.	6.5	52.2	56.9	76
	Gran.		64.9		
A	Gran SB +	5.2	52.4	48.8	56
	Pseud. peat		13.0		
Uninoc	Control	3.5	16.7	61.9	94
	LSD _(0.05)	1.6		8.7	28

SP = seed-placed, SB = side-band

2.3 Irrigated Bean and Field Pea Trials - Materials and Methods

2.3.1 Irrigated Pea and Bean Field Trials - 1998

In 1998, the impact of inoculant formulation for field pea and dry bean production was again conducted in collaboration with Mr. Terry Hogg at the Saskatchewan Irrigation Development Centre. The experiments were established and maintained by the S.I.D.C. whereas our group monitored the plots for nodules development and N fixation at final harvest.

The two separate experiments were established in the spring of 1998 at the Saskatchewan Irrigation Diversification Centre (SIDC) located on the NW12-29-08-W3. The soil, developed on sandy glacial-fluvial and lacustrine deposits, was classified as Bradwell SL-LFS. Although every effort was made to situate the plots on soils low in available N, soils for the dry bean plot contained as much as 50 kg ha⁻¹ in the to 30 cm whereas the soil used for the pea experiment contained as much as 43 kg ha⁻¹ in the top 30 cm.

All seeding operations were conducted by the S.I.D.C. using a small plot six row double disc press drill with two sets of discs. One set of discs was used for seed placement whereas the second set of discs allowed for side band placement of fertilizer and granular inoculants. The treatments were as follows: liquid (A and C); peat-based powder (A and B); clay based granular (A); peat-based granular (B); and an uninoculated control. Inoculants with the same designation, e.g., A, B or C, indicate that identical

Rhizobium strain or strains were used in the different carriers. Inoculants designated A contained a single strain (MicroBio RhizoGen, Saskatoon, SK) whereas inoculants designated B contained a mixture of strains (LiphaTec Inc., Milwaukee, W.I). The liquid inoculant designated C, was supplied by Agrium Biologicals, but was formulated by LiphaTec, Inc.. Liquid C contained a single strain. The granular inoculants were placed either in the seed row or beside (2.5 cm) and below (2.5 cm) the seed row. Flax was used as a control crop for ¹⁵N estimates of N₂ fixation.

All plots received a side band application of a 10-41-12 blend at a rate of 120 kg ha⁻¹ during the seeding operation. Carneval pea was seeded at a target plant population of 80 seeds m⁻² using a 20 cm row spacing. Othello pinto bean, treated with Captan, Methoxychlor and Streptomycin Sulfate prior to seeding, was row crop seeded at a target plant population of 30 seeds m⁻² using a 60 cm row spacing. The treatments were arranged in a randomized complete block design with four replicates. Each treatment consisted of two passes with the drill and measured 2.4 m x 6 m. Individual treatments were spaced on 3 m centers. The trials were seeded on May 7 for pea and May 23 for dry bean and harvested on August 26 for pea and September 30 for dry bean.

Nodule rating of each treatment was determined at flat pod using a protocol developed for pea by Dr. W. Rice, Agriculture and AgriFood Canada, Beaverlodge, Alberta. Plant height was determined by measuring stem length of 5 plants in each individual treatment at the pod fill growth stage. At final harvest, a 1 m2 area was hand harvested from the bean plots for total biomass estimation. No attempt was made to estimate total biomass production of peas due to the difficulty of extracting a small sample from the plots without damaging the remaining plot area. All plots were then direct cut using a small plot combine. For pea the entire treatment area was cut while for dry bean only the two center rows were cut from each treatment. The seed samples were cleaned, weighed and a subsample was used to determine seed weight and nitrogen content. The percentage of N derived from the atmosphere was calculated based on ¹⁵N natural abundance and the difference in nitrogen uptake compared to a flax control.

2.4 Results of the Irrigated Bean and Field Pea Trials - 1998

2.4.1 Results of the Irrigated Bean Field Trials - 1998

In 1998, there was no effect of inoculant formulation on irrigated Othello pinto bean plant stand, yield, seed weight, plant height or nodule rating (Table 48). As well, there was no effect on days to 50% flowering or maturity. All treatments flowered 64 days after seeding and reached maturity 111 days after seeding. It is likely that the lack of response to inoculation occurred as a consequence of relatively high levels of available soil N that limited N fixation in all treatments (Table 49).

Table 48. Agronomic data for the Othello dry bean inoculant formulation evaluation trial grown under irrigation.

Treatment	Yield (kg/ha)	Seed Weight (mg)	Plant Height (cm)	Plant Stand (#/m²)	Nodule Rating
Control	4036	394	47	29	6
Liquid B	4067	404	43	30	6
Liquid A	4060	388	45	29	6
Peat B	4190	394	46	30	6
Peat A	3709	399	47	29	6
Granular B Seed-place	4012	392	45	29	6
Granular B Side-band	3977	393	42	28	7
Granular A Seed-place	3986	390	43	26	7
Granular A Side-band	3906	395	46	28	6
LDS	n.s.	n.s.	n.s.	n.s.	n.s.

0 - no N-fixation potential; 8 - fully effective nodulation

Table 49. Effect of inoculant formulation on estimates of nitrogen fixation (Ndfa) by dry bean and pea, determined using the Natural Abundance method at the Outlook Irrigation Centre, 1998.

Treatment	Formulation	Dry Bean	Field Pea	
a routine in	Tomulation	Nitrogen derived from N fixation (Ndfa)		
A	Liquid	6	10	
В	Liquid	10	7	
A	Peat	9	12	
В	Peat	3	20	
A	Gran. SP	3	19	
A	Gran SB	3	2	
В	Gran SP	3	15	
В	Gran SB	13	8	
Uninoc.	Control	5	6	
	LSD _(0.05)	n.s.	n.s.	

² Means followed by the same letter in each column are not significantly different (L.S.D., p=0.05).

2.4.2 Results of the Irrigated Field Pea Trials - 1998

As was observed for the bean trials conducted in 1998, there was no effect of inoculant formulation on irrigated Carneval pea plant stand, seed weight, plant height or nodule rating (Table 50). The overall plant stand was low for irrigated pea production. Yield was lowest for the control treatment and was significantly increased with the application of all formulations of inoculant. The yield for the liquid and side band granular A applications were higher than the control, although the differences were not considered statistically significant. As well, there was no effect on days to 10% flowering or maturity. All treatments flowered 57 days after seeding and reached maturity 95 days after seeding.

The lack of significant effects of inoculation on the various growth parameters likely reflects the low levels of N fixation achieved in 1998 (Table 49). Relatively low levels of N fixation likely reflect the relatively high levels of available soil N at this site.

Table 50. Agronomic data for the Carneval pea inoculant formulation evaluation trial grown under irrigation.

Treatment	Yield kg ha ⁻ 1	Seed Weight (mg)	Plant Height (cm)	Plant Stand (#/m²)	Nodule Rating ¹
Control	4159 d	238	73	46	6
Liquid B	5149 abc	232	83	46	6
Liquid A	4584 bcd	235	73	50	6
Peat B	5328 abc	233	83	57	6
Peat A	5016 abc	232	84	53	7
Granular B Seed-place	4810 abcd	235	83	44	6
Granular B Side-band	5369 a	228	85	47	6
Granular A Seed-place	5172 abc	233	82	39	7
Granular A Side-band	4470 cd	235	77	43	7

To - no N-fixation potential; 8 - fully effective nodulation

² Means followed by the same letter in each column are not significantly different (L.S.D., p=0.05).

2.5 Dryland Bean Field Trials - Materials and Methods

2.5.1 Dryland Bean Field Trials - 1998

In 1998, Westco. Fertilizers Ltd. contributed \$10,000 towards research in the area of inoculants for dry bean production. It was hoped that the funds would help support the already existing research in the area of inoculant formulation. Together with support from the Crop Development Centre (Dr. Bert Vandenburg), our research group was able to establish three additional experiments at Kenaston, Rosthern and Floral using dry bean (var. Camino) as the test crop. The experiments were seeded on the following dates:

Kenaston – May 21; Rosthern – May 26; and Floral – May 22. Unfortunately, the experiment established at Kenaston had to be abandoned as a consequence of a killing frost and persistent drought.

The experiment at Floral was conducted at the Saskatchewan Pulse Growers (SPG) farm (Dark Brown Chernozemic soil) and the second experiment near Rosthern was conducted at the Seeger Wheeler Farm (Black Chernozemic soil). The Floral site contained 10 kg ha⁻¹ available N in the 0-60 cm depth whereas the soil at Rosthern had 19 kg ha⁻¹ available N to the same depth.

Liquid, peat-based and granular inoculants supplied by MBR and by Liphatech Inc. (granular and peat-based) and Agrium (liquid) were compared using a protocol similar to that used for chickpea, as described in a previous section.

The experiments were conducted using a randomized complete block design consisting of nine treatments, replicated four times. Treatments were similar to those used in related chickpea experiments (see Section 1). The treatments consisted of commercially available inoculants supplied by MicroBio RhizoGen Corp. (MBR), Agrium Biologicals, or Liphatech Inc. and were as follows: 1) seed inoculation using two different peat inoculants (A or B brand) or two different liquid inoculants (A or B brand); 2) soil inoculation, with two granular inoculants (A or B) placed either in the furrow with the seed at planting or side banded 2.5 cm below the seed and 3) a non-inoculated control. Inoculants with the same designation, e.g., A, indicate that identical *Rhizobium* strain or strains were used in the different carriers. Inoculant A contained a single strain, MBW 31 (MicroBio RhizoGen, Saskatoon, SK) whereas inoculant B was a commercial inoculant containing a mixture of strains (LiphaTec Inc., Milwaukee, W.I). The liquid formulation of inoculant B was not available in 1998; hence an experimental liquid formulation (Inoculant C) (Agrium Biologicals Inc., Saskatoon, SK) was used.

Seed inoculation was performed by thoroughly mixing a measured amount (according to manufacturer's recommendations) of peat or liquid inoculant with 1.5 kg seed in plastic bags using 2 ml of 1% gum arabic solution as sticker immediately before seeding. The granular inoculants were applied through a separate box connected to sidebanding discs (adjusted for the appropriate depth). Triple superphosphate (0-45-0) at the rate of 15 kg P₂O₅ ha⁻¹ was applied at planting in the seed row. To avoid contamination, the non-inoculated plots were planted first. In addition, treatments containing the same *Rhizobium* strains were planted consecutively before switching to other treatments to minimize the potential for inadvertently contaminating the treatments. Moreover, the seeding equipment was thoroughly cleaned with a vacuum cleaner and then disinfected with 70% ethanol between each treatment.

Data from the two locations were combined and subject to analysis of variance (SAS Institute).

2.6 Results of the Dryland Bean Field Trials - 1998

Plant biomass, seed yield and harvest index was unaffected by inoculation and inoculant formulation in 1998 (Table 51). However, both seed N concentration and accumulation of N in the seed were positively influenced by inoculant application. In

particular, application of liquid C or granular A placed in the seed row and applied in a sideband, enhanced seed N concentration. The peat based powder A applied to the seed, which contained the same rhizobial strain similarly enhanced seed N concentration.

The accumulation of N in the seed reflected trends similar to that observed for seed N concentration (Table 51). In particular, peat based powder A and granular A applied in the seed row performed well in terms of promoting N accumulation in the seed. However, these treatments were not statistically different from any of the other inoculant treatments although they both performed better than the uninoculated control.

Table 51. Biomass, seed yield, seed N concentration and seed N uptake and harvest index at Rosthern and Floral (combined site analysis) for dryland bean (var. CDC Camino), 1998.

Inoculant [†]	Biomass (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Harvest Index	Seed N Concentration (%)	Seed N Accumulation (kg ha ⁻¹)
Non-inoculated	2746	1171	0.43	3.3	39.6
Liquid A	2758	1202	0.45	3.2	40.5
Liquid C	2954	1193	0.42	3.8	45.8
Peat A	2866	1328	0.47	3.7	49.0
Peat B	2751	1192	0.44	3.6	43.8
Gran A with seed	2980	1288	0.45	3.9	51.0
Gran A 2.5 cm bs	2734	1141	0.43	3.8	47.3
Gran B with seed	2791	1190	0.43	3.5	42.5
Gran B 2.5 cm bs	2517	1063	0.43	3.3	36.1
LSD _(0.05)	n.s.	n.s.	n.s.	0.2	11.8

Gran = granular, ws = with seed.

Beans are known to be poor N-fixers and our data confirms that CDC Camino, grown under dryland conditions, fixed only moderate amounts of N (Table 52). For example, uninoculated beans derived 15% of the N in the seed via N fixation, presumably through the contribution of indigenous rhizobia in the soil. Inoculation improved these levels to a maximum of only 48%, with liquid C. This level of N fixation was not significantly different from that achieved when using peat based powder A, or granular A applied as a side band treatment. Interestingly, granular B did not perform well in these experiments with regards to N fixation.

The total quantity of N fixed followed a similar trend as was observed for %Ndfa (Table 52). Specifically, liquid C and peat-based powder A or granular A in the seed row and applied as a sideband treatment resulted in maximum accumulation of fixed N. As was observed for the estimates of %Ndfa, granular B did not perform as well as the others inoculants used in these experiments. Similarly, strain A, formulated as a liquid inoculant performed poorly in terms of promoting N fixation. The difference in response of the beans to liquid A and liquid B indicate that either formulation or strain differences can contribute to the success of an inoculant. Moreover, the fact that liquid C performed well in this experiment indicates that in some instances, liquid inoculants can be as

effective as other formulations. It is important to note that symbiotic N fixation did not contribute large amounts of N to the N nutrition of the crop. Even the most effective treatment contributed less than 20 kg N ha⁻¹. These relatively low levels of N fixation suggest that bean may benefit from the application of external N sources.

Table 52. Nitrogen derived from fixation (%Ndfa) and accumulation of fixed N (kg ha⁻¹) for dryland bean (var. CDC Camino), grown at Rosthern and Floral (combined site analysis) in 1998.

Inoculant [†]	Ndfa (%)	Fixed N (kg ha ⁻¹)
Non-inoculated	15	4.4
Liquid A	16	5.7
Liquid C	48	21.5
Peat A	37	18.2
Peat B	26	10.9
Gran A with seed	34	16.1
Gran A 2.5 cm bs	36	17.0
Gran B with seed	25	10.6
Gran B 2.5 cm bs	20	6.6
LSD _(0.05)	13	7.4

Gran = granular, ws = with seed.

2.7 Summary of the Inoculant Formulation for Dryland Bean, and Irrigated Bean and Pea Experiments

Experiments conducted under both irrigation and dryland conditions support the contention that beans are not efficient N fixers. Under irrigated conditions used in these experiments, it is likely that N fixation was limited by the relatively high levels of available N in the. However, even when the soil N levels were considerably lower, as was the case for the dryland bean experiments, the levels of N₂ fixation remained relatively low.

Although levels of N₂ fixation were relatively low in the irrigated bean experiments, it is interesting to note that the highest seed yields typically were associated with the granular inoculant treatments, although treatment differences often were not considered statistically significant. These results are in keeping with reports by others who have similarly observed that granular inoculants are often as good as, and frequently better, than liquid and peat-based powders (e.g., Brockwell et al., 1980, 1985).

Data from irrigated field pea trials similarly suggest that inoculation using a granular formulation was successful in terms of promoting seed yield. It is important to note, however, that the other formulations (i.e., liquid and peat), in some instances, performed as well as the granular products. This observation lends further support to the assertion that, given ideal conditions, the currently available inoculant formulations can be equally capable of promoting similar levels of nodulation and subsequent seed yield.

In general, although placement of the granular products apparently influenced the success of the inoculation treatment, the effects of placement (i.e., sideband versus seed row placement) were inconsistent and unpredictable. Therefore, we concluded that either placement can lead to successful nodulation, as long as the band is in close proximity to the emerging root.

3. The Efficacy of Fall Banded Granular Inoculants

Although newer seeding equipment is being developed to accommodate application of multiple inputs (i.e., seed, fertilizer and inoculant in three separate boxes), current seeding equipment is typically limited to two compartments. As a consequence, producers are unable to apply granular inoculants during the seeding/fertilizing operation. As an alternative, we investigated the possibility of banding granular inoculants in the fall, prior to seeding.

3.1 Materials and Methods

Field experiments were conducted over a three year period to examine the impact of fall banded granular inoculant on the nodulation, N₂ fixation and yield of chickpea and bean. The first experiments were established in the fall of 1996 and were located at Clavet and Saskatoon (Kernen Crop Research Farm) using desi-type chickpea (var. Myles) as the test crop. Soils at both sites are Dark Brown Chernozems and previously had been cropped to cereals. Soil tests indicated relatively low levels of available N at Saskatoon (23 kg N ha⁻¹ to 60 cm) and Clavet (21 kg N ha⁻¹ to 60 cm) in the spring of 1997, prior to seeding.

The 1998 field plots were established in the fall of 1997 and were located at Young using desi-type chickpea (var. Myles), and beans (var. Othello) as the test crops. The soil at Young is a Dark Brown Chernozem and previously had been cropped to flax. Spring soil tests indicated the presence of 25 kg ha⁻¹ of available soil N (to 60 cm) at the Young site.

The 1999 field season represented the third and final year of these experiments. The 1999 field experiments were conducted at two locations (Outlook and Clavet). Kabuli-type chickpea (var. Sanford) was used as the test crop at Outlook whereas bean (var. CDC Camino) was used as the test crop at Clavet. At Clavet, spring soil tests indicated 24 kg N ha⁻¹ to 60 cm whereas spring soil tests at Outlook indicated 18 kg N ha⁻¹ to 60 cm.

The experiments were conducted using a split plot experimental design arranged in a randomized complete block with four replicates. Two different granular soil implant inoculants (supplied by MBR (BCF32) and Liphatech Inc.) were applied as fall banded treatments. The inoculants were banded in rows at a depth of 8 cm prior to the first snow. Specifically, in 1996, granular inoculants were banded on October 18 at Kernen and November 5 at Clavet. In 1997, the inoculants were banded in rows at a depth of 8 cm on October 31. In 1998, the inoculants were banded at Outlook and Clavet on October 28 and October 30, respectively.

In the spring of the following year, the test crops were seeded into the granular treatments at right angles to the original fall granular inoculant placement. The fall banded treatment was compared to spring granular inoculants (supplied by MBR (i.e., inoculant A) and Liphatech Inc. (inoculant B)) placed in the seed row, seed applied peat-based inoculant (both MBR (A) and Liphatech Inc. (B)), seed applied liquid inoculant (both MBR (A) and Liphatech Inc. (B)) and an uninoculated control. Inoculants followed

by the same letter designation (i.e., A or B) indicate formulations that contain the same strain, or mixture of strains. Flax (var. McGregor) served as a non-fixing reference crop for ¹⁵N natural abundance estimation of N₂ fixation. Thus, each experiment consisted of ten treatments, replicated four times.

In 1997, a slightly different set of treatments were used at Clavet. Treatments were limited to the MBR products (inoculant A) applied either at the recommended application rate, or at twice the recommended rate of application. The experimental design used in 1999 for the chickpea experiment also differed from previous years in that it did not include a liquid Liphatech Inc. inoculant. We made every effort to obtain a liquid inoculant for chickpea from Liphatech Inc.; however, this product is not produced commercially and company representatives were reluctant to produce a non-commercial product for experimental purposes. After considerable discussion, it was eventually decided to delete the liquid Liphatech Inc.product from the experimental design.

Seeding in the spring of 1997 occurred on May 27 at both Kernen and Clavet. In 1998, seeding of the chickpea was accomplished on May 13, whereas beans were seeded on May 25. In 1999, seeding was accomplished on May 7 at Outlook, and May 26 at Clavet.

In 1999, cool and wet conditions delayed emergence of the bean crop at Clavet and some seeds succumbed to rot. Although the stand that eventually emerged was considered thin, we maintained the experiment and harvested the plots for final seed yield and N_2 fixation. The chickpea crop was very responsive to the moisture conditions experienced in 1999. Like many pulse crops grown in 1999, however, there was excessive vegetative growth with minimal seed set. Although this crop was seeded in early May, it continued to flower as late as mid-August and many of the treatments were not fully mature at first frost.

A final harvest was conducted at all sites, by hand (1 m²). Final seed yield and harvest index, was determined. Biological N₂ fixation was determined using the ¹⁵N natural abundance technique.

Where appropriate, data from different sites were combined and an analysis of variance was performed (SAS Institute). Analysis of the data indicated significant location by treatment effects. In particular, 1999 differed from other years, presumably due to the unusually wet and cool conditions experienced during the final year of experimentation. Thus, the data are presented individually, by year, and by crop.

3.2 Results of the Chickpea Field Trials

3.2.1 Results of the Chickpea Field Trials - Clavet, 1997

In the first year of fall applied granular inoculant experiments, it was not known if fall inoculation would be successful. Furthermore, we did not know if the success of fall applications would be dependent on the rate of inoculant applied. Therefore, we included treatments designed to examine the impact of application rate on inoculation success. Interestingly, seed yield typically was unaffected by the rate of application of the granular A inoculant (Table 53). Moreover, the highest seed yields were achieved when the

rhizobia were applied to the seed as a peat based powder formulation. However, although the peat-based powder enhanced seed yields relative to the control, differences between the inoculant treatments were not considered statistically significant. Biomass yields at maturity followed a pattern similar to that observed for seed yields.

Relatively high harvest index values suggest that a greater proportion of plant resources was directed to producing seed yield, rather than vegetative yield (Table 53). This partitioning was apparently exaggerated in the control and the liquid inoculant treatments, suggesting that vegetative production may have been somewhat limited in these treatments.

Table 53. The impact of inoculant formulation and time of application on seed and biomass yield and harvest index of desi chickpea at Clavet, 1997.

Treatment	Seed Yield (kg ha ⁻¹)	Biomass Yield (kg ha ⁻¹)	Harvest Index
Granular (A) Fall Applied (recommended rate)	988	1660	0.59
Granular (A) Fall Applied (2X recommended rate)	905	1577	0.58
Granular (A) Spring (recommended rate)	1072	1645	0.66
Granular (A) Spring (2X recommended rate)	945	1500	0.65
Peat (A) recom'd rate	1140	1762	0.65
Liquid (A) recom'd rate	1042	1542	0.71
Uninoc. Control	815	1225	0.68
LSD _(0.05)	308	543	0.07

Inoculant rate had little impact on the success of granular inoculation, applied either in the fall or at seeding in terms of N_2 fixation (Table 54). Interestingly, the highest estimate of N_2 fixation was associated with the peat based powder treatment although this level of fixation was not significantly different from levels achieved using either fall applied treatments (i.e., 1X and 2X the recommended rate) or the spring applied granular treatment at twice the recommended rate, or the liquid treatment. All treatments enhanced N_2 fixation relative to the uninoculated control.

It is interesting to note that N_2 fixation also was detected in the uninoculated control plots (Table 54). This observation was not anticipated as it is generally assumed that there are no indigenous *Rhizobium* capable of causing chickpea nodulation in our Saskatchewan soils. Several possibilities exist to explain the occurrence of nodules and N fixation in the control plots. Firstly, it is possible that the seed lot itself was contaminated with *Rhizobium* and living bacteria were present on the seed coat of the

untreated seed. Secondly, although precautions were taken to avoid contamination between plots, including wiping down the seeding equipment and delivery tubes with 70% alcohol, it is possible that some contamination occurred during the seeding operation.

Table 54. The impact of inoculant formulation and time of application on seed protein and nitrogen fixation (%Ndfa) of desi chickpea at Clavet, 1997.

Treatment	Seed Protein	Ndfa
	(%)	(%)
Granular (A) Fall Applied (recommended rate)	20.0	37.2
Granular (A) Fall Applied (2X recommended rate)	20.4	40.0
Granular (A) Spring (recommended rate)	20.4	32.2
Granular (A) Spring (2X recommended rate)	19.8	36.0
Peat (A) recom'd rate	22.4	50.2
Liquid (A) recom'd rate	19.9	48.5
Uninoc. Control	17.8	30.0
LSD _(0.05)	2.0	17.5

3.2.2 Results of the Chickpea Field Trials - Kernen, 1997

Results from the site at Kernen in 1997 suggest that the fall applied inoculants (both A and B) successfully promoted both seed and total biomass yield (Table 55). Surprisingly, the spring applied granular A did not perform well and both seed and biomass yields associated with the spring applied treatment were similar to the control. We are unable to adequately explain this apparent inoculant failure. As was observed at Clavet, harvest indices in 1997 were relatively high, indicating that seed production was favored over vegetative growth.

Table 55. The impact of inoculant formulation and time of application on seed and biomass yield and harvest index of desi chickpea at Clavet, 1997.

Treatment	Seed Yield (kg ha ⁻¹)	Biomass Yield (kg ha ⁻¹)	Harvest Index
Granular (A) Fall Applied	1393 a	2210 a	0.63
Granular (B) Fall Applied	1278 ab	2062 ab	0.62
Liquid (A)	1085 bc	1892 abc	0.58
Peat (A)	1375 a	2292 a	0.61
Granular (A) Spring	962 c	1595 bc	0.62
Liquid (B)	1158 abc	1947 abc	0.62
Peat (B)	1020 с	1555 с	0.69
Granular (B) Spring	1370 a	2210 a	0.62
Uninoc. Control	930 с	1530 с	0.63
LSD _(0.05)	257	503	n.s.

As was observed for the yield parameters, fall application of granular inoculants enhanced both N₂ fixation and protein production (Table 56). Indeed both fall granular inoculant treatments (A and B) performed as well, or better, than the spring applied counterparts in terms of promoting N₂ fixation. As was observed at the Clavet site, low levels of N fixation were associated with the uninoculated control, which suggests that some contamination occurred.

Table 56. The impact of inoculant formulation and time of application on seed protein and nitrogen fixation (%Ndfa) of desi chickpea at Kernen, 1997.

Treatment	Seed Protein (%)	Ndfa (%)
Granular (A) Fall Applied	21.7	51
Granular (B) Fall Applied	19.6	56
Liquid (A)	18.2	36
Peat (A)	21.3	38
Granular (A) Spring	17.2	32
Liquid (B)	19.2	36
Peat (B)	18.0	32
Granular (B) Spring	21.1	57
Uninoc. Control	18.8	20
LSD _(0.05)	2.7	17

3.2.3 Results of the Chickpea Field Trials - Young, 1998

The chickpea trial conducted in 1998 served as the basis to an undergraduate thesis project conducted by Ms. Cindy Fedirko. As a consequence of Ms. Fedirko's involvement, additional measurements were taken in 1998 that were not repeated in other years. Thus, a complete discussion of the 1998 chickpea field trial, as conducted and reported by Ms. Fedirko, is included in this report.

Fall-applied granular inoculants produced significantly higher numbers of nodules on the lateral root positions whereas nodules on the primary root made a relatively small contribution to total nodule mass (Table 57). When peat or liquid formulationswere applied to the seed, the nodule numbers were highest in the crown and primary root regions. Although the number of nodules typically were highest on the lateral roots when granular inoculants were used, nodule dry weights associated with the granular inoculant formulation were significantly lower than where either the liquid of the peat based powder formulations were used. These data suggest that soil implant inoculation produces many smaller nodules that are well distributed on the lateral root system whereas seed applied formulations produce nodules that are larger and are fewer in

number. It is expected that the number, size and placement of the nodules may have an impact on the amount of nitrogen fixed over the growing season. Nodulation, although limited, was observed in the control plots.

Table 57. The impact of inoculant treatment on development of chickpea nodules.

	Numbe	Number and Location of Nodules		
Treatment	Lateral Region	Crown Region	Primary Root Region	(mg plant ⁻¹)
Granular (A) Fall Applied	80a	5cd	3.2abc	712bc
Granular (B) Fall Applied	51abc	5ed	6.0abc	822abc
Liquid (A)	38bcd	33ab	3abc	1195ab
Peat (A)	44bcd	35a	7abc	1538a
Granular (A) Spring applied	25cd	17bc	9ab	1212ab
Liquid (B)	28cd	30ab	6abc	1128ab
Peat (B)	24cd	40a	11a	1168ab
Granular (B) Spring applied	72ab	12cd	2bc	995ab
Uninoc. Control	12d	Od	0c	230c

² Means followed by the same letter in each column are not significantly different (LSD_(0.05)).

Irrespective of inoculant formulation, N₂ fixation was enhanced by all treatments relative to the control (Table 58). Little difference existed between the different inoculant treatments in terms of the percentage of N derived from fixation. It is particularly interesting to note that excellent levels of N₂ fixation were achieved when the granular inoculants were applied as a fall banded treatment. Clearly, application of the granular inoculants in the fall did not compromise the ability of the *Rhizobium* to establish effective nodulation. Seed protein levels also were favorably enhanced by the inoculant treatments and there were no significant differences between the various treatments.

Table 58. The impact of inoculant treatment on N₂ fixation and final seed protein content.

Treatment	Nitrogen derived from fixation (%)	Seed Protein (%)
Granular (A) Fall Applied	69ab	23.1a
Granular (B) Fall Applied	72a	21.6a
Liquid (A)	71ab	22.0a
Peat (A)	70ab	21.8a
Granular (A) Spring applied	69ab	21.3a
Liquid (B)	66b	22.5a
Peat (B)	67ab	22.7a
Granular (B) Spring applied	69ab	21.4a
Uninoc. Control	6c	18.0b

² Means followed by the same letter in each column are not significantly different (L.S.D., p=0.05).

Few differences existed between the different inoculant formulations in terms of final biomass production and seed yield of desi-type chickpea (Table 59). Surprisingly, the granular B did not perform as well as granular A in terms of seed yield, whether applied in the fall or at seeding. Differences between granular A and granular B applied in the spring were not considered statistically significant. Differences between the two granular products are not consistent with results of related experiments (i.e., Section 1 of this report) conducted at other locations. Currently, we are unable to explain this apparently anomalous result.

Table 59. The impact of inoculant treatment on N₂ fixation and final seed protein content.

Treatment	Total Biomass Production (kg ha ⁻¹)	Seed Yield (kg ha ⁻¹)
Granular (A) Fall Applied	5852a	2784a
Granular (B) Fall Applied	4588c	2390c
Liquid (A)	5235abc	2670abc
Peat (A)	5448ab	2757ab
Granular (A) Spring applied	5210abc	2574abc
Liquid (B)	5573ab	2810a
Peat (B)	5685a	2816a
Granular (B) Spring applied	5015bc	2479bc
Uninoc. Control	2905d	1462d

² Means followed by the same letter in each column are not significantly different (LSD_(0.05)).

3.2.4 Results of the Chickpea Field Trials - Outlook, 1999

Relatively wet, cool growing season promoted excessive vegetative growth at Outlook in 1999 and some treatments did not fully mature before the first fall frost. Thus, interpretation of the data is difficult. Foe example, low seed yields may reflect a relatively unsuccessful inoculant treatment or, alternatively, low yields may reflect the fact that the crop had not fully matured and the seed was underweight and green.

In general, seed yields were very low relative to the vegetative production and, as a consequence, harvest index values were exceptionally low (Table 60). Although strict interpretation of the data is difficult, final seed yield suggests that the fall applied inoculant treatments were effective. Visual assessments of the treatments throughout the

growing season clearly indicated that the fall applied granular inoculant effectively promoted crop growth.

Interestingly, some treatments did not appear to enhance seed yield relative to the uninoculated control (e.g., granular products applied in the spring) (Table 60). However, examination of the data reveals that although these treatments did not enhance seed yield, biomass production was promoted. Thus, the low seed yields occurred, in part, as a consequence of the success of the granular inoculant treatments. Chickpea has an indeterminate growth habit, and normally requires a moisture or N stress to encourage seed production. In the absence of either stress, crop growth continued to the extent that maturity was not reached prior to the first killing frost.

Interestingly, the highest seed yields were achieved when the granular inoculants were applied in the fall (Table 60). Moreover, these relatively high seed yields also were associated with relatively high biomass production. These results suggest that the fall inoculant treatment successfully promoted N₂ fixation and consequent growth, but the plants were still able to produce more mature seed than the control treatment. It is possible that the mechanisms that controlled yield in the spring applied granular inoculant treatments were different than those associated with the fall applied granular inoculant treatments. However, we are unable to provide a satisfactory explanation for these different responses.

Table 60. The impact of inoculant formulation and time of application on seed and biomass yield and harvest index of desi chickpea at Outlook, 1999.

Treatment	Seed Yield (kg ha ⁻¹)	Biomass Yield (kg ha ⁻¹)	Harvest Index
Granular (A) Fall Applied	1145	5550	0.21
Granular (B) Fall Applied	1187	4472	0.27
Liquid (A)	476	5622	0.09
Peat (A)	674	4528	0.16
Granular (A) Spring Seed-placed	741	5052	0.15
Granular (A) Spring Sidebanded	883	5114	0.19
Peat (B)	1250	5672	0.24
Granular (B) Spring Seed-placed	607	4614	0.14
Granular (B) Spring Sidebanded	497	5074	0.10
Uninoc. Control	678	2908	0.24
LSD _(0.05)	420	1687	0.09

Exceptionally high levels of N_2 fixation were achieved at Outlook in 1999 (Table 61). Apparently, the same conditions that enhanced vegetative growth also promoted N_2 fixation. This observation is not surprising because actively growing plants are able to allocate significant carbon resources to the nodules, effectively promoting N_2 fixation.

Seed protein levels were highly variable at Outlook in 1999. The variability in the protein levels likely reflects the fact that treatments differed in the degree of maturity at the time of the first killing frost.

Table 61. The impact of inoculant formulation and time of application on seed protein and N₂ fixation (%Ndfa) of desi chickpea at Outlook, 1999.

Treatment	Seed Protein	Ndfa
	(%)	(%)
Granular (A) Fall Applied	22.0	81
Granular (B) Fall Applied	20.9	78
Liquid (A)	24.3	65
Peat (A)	24.4	58
Granular (A) Spring Seed-placed	24.8	71
Granular (A) Spring Sidebanded	23.9	55
Peat (B)	23.4	66
Granular (B) Spring Seed-placed	22.6	63
Granular (B) Spring Sidebanded	21.1	64
Uninoc. Control	15.6	. 21
LSD _(0.05)	3.2	20

3.2.5 Results of the Bean Field Trials - Young, 1998

Bean seed and biomass yields in 1998 at Young were relatively poor (Table 62) and may reflect a poor adaptation of dry bean to this particular soil zone (i.e., Dark Brown soil zone). Although the climatic conditions were conducive to early germination and emergence, growth beyond the seedling stage was severely restricted and plants did not reach heights that would have allowed mechanical harvesting. Interestingly, the harvest index values indicate that the allocation of dry matter to biomass production was

not disproportionate, providing further evidence that the environment exerted strong controls on bean growth at this site, and limited both biomass and seed yield production.

Although dryland bean yields were limited at Young in 1998, application of granular inoculants A and B, applied in the spring, served to enhance seed yields relative to the uninoculated control (Table 62). Of particular interest is the observation that the fall granular inoculant failed to enhance seed yield relative to the control. In contrast, total biomass production was enhanced by the application of granular inoculants, but only when these inoculants were applied at seeding. The failure of the fall inoculant treatment to enhance bean growth and yield contrasts the success achieved when chickpea was used as the test crop. These results suggest that the success, or failure, of a fall banded granular inoculant treatment may be dependent on the *Rhizobium* strain, the crop, or both.

Table 62. The impact of inoculant formulation and time of application on seed and biomass yield and harvest index of dryland bean at Young, 1998.

Treatment	Seed Yield (kg ha ⁻¹)	Biomass Yield (kg ha ⁻¹)	Harvest Index
Granular (A) Fall Applied	398 bc	1092 bc	0.36
Granular (B) Fall Applied	365 с	1012 bc	0.37
Liquid (A)	436 bc	1078 bc	0.41
Peat (A)	379 с	947 с	0.41
Granular (A) Spring	648 a	1625 a	0.42
Liquid (C)	528 ab	1163 bc	0.47
Peat (B)	434 bc	1005 с	0.46
Granular (B) Spring	621 a	1347 ab	0.47
Uninoc. Control	415 bc	1092 bc	0.39
LSD _(0.05)	142	341	n.s.

Bean seed protein levels were relatively low in 1998 but, surprisingly, the percent N derived from fixation (%Ndfa) was relatively high, even in the uninoculated control treatment (Table 63). In light of the poor bean growth and yields at this location, it is possible that the relatively high %Ndfa values occurred as an artifact of the technique used to estimate N₂ fixation, rather than due to real effects on N₂ fixation itself. Estimates that the uninoculated control apparently obtained as much as 48% of the crop N via N₂ fixation are suspect, and lends further support to this possibility.

Table 63. The impact of inoculant formulation and time of application on seed protein and nitrogen fixation of dryland bean at Young, 1998.

Treatment	Seed Protein	Ndfa
	(%)	(%)
Granular (A) Fall Applied	11.8 с	52 cd
Granular (B) Fall Applied	12.4 bc	64 b
Liquid (A)	12.7 bc	60 bc
Peat (A)	13.0 bc	59 bc
Granular (A) Spring	12.6 bc	84 a
Liquid (C)	12.4 bc	52 cd
Peat (B)	13.2 Ъ	48 d
Granular (B) Spring	15.3 a	77 a
Uninoc. Control	13.2 b	48 d
LSD _(0.05)	1.2	11

3.2.6 Results of the Bean Field Trials - Clavet, 1999

In 1999, cool and wet conditions delayed emergence of the bean crop at Clavet and some seeds succumbed to rot. Moreover, emergence was very uneven and some seedlings took as long as three weeks to fully emerge, at which time they appeared to be stunted and weak. Although the stand that eventually emerged was thin, we maintained the experiment and harvested the plots at maturity.

Bean seed yields were low in 1999, but given the slow emergence and early establishment of the crop, coupled with the relatively thin stand, we were pleasantly surprised with the final seed yields (Table 64). The highest seed yields were achieved when the granular A product was applied with the seed at the time of seeding. This yield, however, was not statistically different than that achieved when the granular inoculants were banded in the fall of the previous year.

It is interesting to note that the granular B product, applied at seeding in the seed row, was not significantly different than the control (Table 64). This product had initially

been procured for field experiments conducted in 1998 and had been stored over the winter months at 5°C. It had not been our intention to reuse this product. However, in 1999 there was a severe shortage of granular peat products and, for the first time, we were unable to procure a granular peat inoculant for beans, even from the manufacturer for research purposes. As an alternative, we tested the stored product using standard plate counts and we determined that the number of rhizobia per gram of product had not declined significantly during storage. Moreover, the rhizobial populations had remained within the range initially guaranteed by the manufacturer. However, poor seed and biomass yield responses to this stored product suggest that although viability (as determined in the laboratory) may not have been impaired by storage, the ability of the *Rhizobium* to multiply and infect the plant roots appeared to have been compromised. These results should be taken as a cautionary note for producers who want to use product that has been stored past the expiration date.

Table 64. The impact of inoculant formulation and time of application on seed and final biomass yield and harvest index of dryland bean at Clavet, 1999.

Treatment	Seed Yield (kg ha ⁻¹)	Biomass Yield (kg ha ⁻¹)	Harvest Index
Granular (A) Fall Applied	1271 ab	3413 ab	0.37 a
Granular (B) Fall Applied	1226 ab	3329 ab	0.37 a
Liquid (A)	1122 ab	2757 ь	0.40 a
Peat (A)	968 b	2611 b	0.37 a
Granular (A) Spring	1530 a	4120 a	0.37 a
Liquid (C)	1068 b	3028 ab	0.36 ab
Peat (B)	1114 ab	2954 ab	0.38 a
Granular (B) Spring	1075 b	2770 Ь	0.39 a
Uninoc. Control	960 b	3537 ab	0.30 b
LSD _(0.05)	450	1242	0.06

In 1999 at the Clavet site, the highest levels of N₂ fixation and seed protein levels occurred when granular A inoculant was applied at seeding in the seed row (Table 65). The spring applied granular inoculant resulted in significantly higher levels of N₂ fixation as compared to the same granular product applied as a fall treatment. Indeed, the fall applied granular A product resulted in the lowest levels of N₂ fixation and did not enhance N₂ fixation relative to the uninoculated control. Interestingly, the fall applied granular B product resulted in N₂ fixation levels similar (i.e., not statistically different) to that achieved with the spring granular A product. These results suggest that some

rhizobial strains may be more effective in terms of long term survival and we may have to assess the survival of each strain independently.

Table 65. The impact of inoculant formulation and time of application on seed protein and nitrogen fixation (%Ndfa) of dryland bean at Clavet, 1999.

Treatment	Seed Protein (%)	Ndfa (%)
Granular (A) Fall Applied	14.4 abcd	37 c
Granular (B) Fall Applied	13.9 bcd	51 abc
Liquid (A)	15.7 ab	45 bc
Peat (A)	15.5 abc	38 c
Granular (A) Spring	15.9 a	63a
Liquid (C)	13.5 d	41 bc
Peat (B)	13.4 d	54 ab
Granular (B) Spring	15.7 ab	52 abc
Uninoc. Control	13.7 cd	40 bc
LSD _(0.05)	1.8	16

3.3 Summary of Fall Applied Granular Inoculant Experiments

Results from the chickpea studies are encouraging and suggest that fall banding of granular products may result in successful nodulation and N₂ fixation in chickpea. These results indicate that the chickpea *Rhizobium* used in these experiments were capable of surviving the winter. Moreover, the positioning of the granular inoculant bands at right angles to the seed rows resulted in excellent nodulation in the subsequent chickpea crop. It is important to recognize that these results may not apply for all *Rhizobium* strains and it is urged that caution be used in interpreting the practical significance of these results.

In contrast to the results from the chickpea experiments, experiments with dry bean indicated that fall application of granular inoculants was typically unsuccessful. It is not known if the failure of a fall banded granular inoculant treatment was related to the *Rhizobium* strain, the crop, or both. For example, the inoculant failure may have occurred because the bean *Rhizobium* were unable to survive the winter conditions. Alternatively, the rooting pattern of the bean plants may have restricted contact between the inoculant bands applied in the fall at right angles to the seed row, thereby limited nodulation. In contrast, granular inoculants applied in the furrow at seeding may have been better positioned to encourage contact between the emerging bean roots and the inoculant granules.

Although the possibility may exist to promote nodulation of pulses by applying granular inoculant s in the fall, inconsistent results (i.e., between sites and with different crops) suggest that this practice is not risk free. Therefore, we have concluded that fall banding of granular inoculants should not be actively promoted as an effective alternative to spring applications without further investigation.

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(d) PROJECT PERSONNEL

	Time	Wages
Bev Miller, Technical Assistant	50%	50%
Cindy Fedirko, Summer Assistant	100%	100%
Jayam Booth, Summer Assistant	75%	75%
Kevin Farden, Summer Assistant	50%	50%
Stephen Kyei-Boahen ^a , Ph.D. Student	100%	0%

^aMr. Kyei-Boahen is supported by an external scholarship.

(e) EQUIPMENT - none.

- (f) PROJECT DEVELOPED MATERIALS—The Principle Investigator (Dr. Fran Walley) has been involved in numerous extension activities (producer meetings and field tours) in which information regarding the use of granular inoculants has been discussed. At several of these meetings, proceedings were supplied. In addition, Dr. Walley has submitted articles to the Saskatchewan Pulse Growers for publication in their newsletter.
- (g) PROJECT PHOTOS Several photos were taken during the course of this study, demonstrating crop development and root nodule development. These photos are available, on request.
- (h) ACKNOWLEDGMENTS The financial support from ADF was acknowledged at all presentations and in all publications relating to this project. The following list outlines the various proceeding, reports and presentations that have been made:

Proceedings and Reports

- F. Walley. 1999. Inoculants and Pulse Crop Inoculation. Proceedings of the Dryland Cropping Systems Conference, p. 25-31, March 4-5, Moose Jaw, SK.
- T. Nleya, F. Walley and A. Vandenburg. 1998. Cultivar and Inoculant Placement Effects on Nodulation and Yield of Dry Bean. Proceedings of the Pulse Crops Research Workshop, p.21-22, Saskatoon, Saskatchewan.
- S. Kyei-Boahen, F.L. Walley, and A.E. Slinkard. 1998. Placement of Granular Inoculant for Chickpea. Proceedings of the Pulse Crops Research Workshop, p.23-24, Saskatoon, Saskatchewan.
- T.M. Nleya, F.L. Walley and A. Vandenburg. 1999. Nitrogen Fixation in Dry Bean as Influenced by Cultivar and Inoculation Method. <u>Proceedings of the 1999 Soils and Crops Workshop</u>, p. 308-313, Saskatoon, Saskatchewan.

F. Walley. 1998. Inoculating Chickpeas. The Saskatchewan Pulse Grower vol. 14, issue 1:14.

F. Walley. 1998. New Opportunities in Pulse Inoculation. Saskatchewan Agriculture and Food Website (http://eru.usask.ca/scripts/research/article.ide?id=30 (19/12/98 12:38 AM)).

Extension Activities

Field Tour "Pulse Crop Inoculation", at the "Harvesting Water – Dryland Cropping Systems" Field Day, sponsored by SPARC/Wheatland Conservation Area, Inc., Swift Current, July 9, 1998.

Presentation on "Inoculants in Pulse Crops – What's New?", at the Crop Management Forum – Cultivating New Ideas, sponsored by the Canadian Association of Agri-Retailers, Saskatoon, November 5, 1998.

Presentation on "Update on Inoculants and Fertilizer Management for Pulse Crops", at the 1998 Extension Agrologists Update, sponsored by Saskatchewan Agriculture and Food Extension Branch, Saskatcon, December 10, 1998.

Presentation on "Growing Chickpeas in Saskatchewan", sponsored by the Saskatchewan Wheat Pool, Craik, January 7, 1999.

Presentation on "Granular Inoculants", at the Western Canadian Crop Production Show 1999 Information Theatre, sponsored by the Western Canadian Crop Production Show, Saskatoon, January 12, 1999.

Presentation on "Inoculating and Fertilizing Pulse Crops", at the Saskatchewan Pulse Growers Information Days, sponsored by the Saskatchewan Pulse Growers and Saskatchewan Agriculture and Food, Swift Current, March 3, 1999.

Presentation on "Inoculants", at the 1999 Dryland Cropping Systems Conference, sponsored by Saskatchewan Agriculture and Food, SPARC, District #8 ADD Board, PFRA, Agri-Food Innovation Fund, REDA, Moose Jaw, March 5, 1999.

Presentation on "Making the Most of Inoculants", at the Farmer Agronomy School, sponsored by Pioneer Co-op Agro Centre, Swift Current, March 10, 1999.

Presentation on "Summary of Pulse Crop Inoculation Studies", at the 1999 Industry Update, sponsored by the Saskatchewan Agriculture and Food Extension Services Branch, Saskatoon, January 28, 1999.

Presentation on "Inoculating and Fertilizing Pulse Crops", at the Farmer Agronomy School, sponsored by Pioneer Co-op Agro Centre, Swift Current, March 1, 2000.

Presentation on "Inoculating and Fertilizing Pulse Crops", at the Farmer Agronomy School, sponsored by Pioneer Co-op Agro Centre, Swift Current, March 15, 2000.

Presentation on "Inoculating and Fertilizing Pulse Crops", at the the Agro Management Forum, sponsored by Westco and the Saskatchewan Wheat Pool, Beatty, March 3, 2000.

Presentation on "Inoculating and Fertilizing Pulse Crops", at the the Agro Management Forum, sponsored by Westco and the Saskatchewan Wheat Pool, North Battleford, March 3, 2000.

(i) EXPENSE STATEMENT - To be provided by the University of Saskatchewan.